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TEMPERATURE, WIND, HUMIDITY AND EVAPORATION IN AGRICULTURAL METEOROLOGY[1]

BY J. W. HOPKINS[2] AND MABEL F. JAMES[3]

Abstract

A statistical study has been made of evaporation and allied meteorological observations for a period of approximately 20 years at each of two stations, situated in Montana and New Mexico respectively.

The average values, over this period, for May, June, July and August of all four meteorological quantities showed a seasonal trend. At both stations July was the month of maximum average temperature, evaporation and vapor pressure. Average wind velocity was greatest at both stations in May, decreasing progressively throughout the summer.

In general the extent of evaporation was markedly influenced by wind and vapor pressure as well as by temperature. Annual variations in evaporation during a specified calendar month were found to be correlated with temperature differences, but here also there was a significant additional effect of wind and vapor pressure. Evaporation measurements should therefore supplement temperature records in agricultural meteorological studies.

Introduction

It is generally believed that the injurious effects of drought upon agricultural crop yields are intensified by high temperatures. These by increasing evaporation might be expected to accelerate drying-out of the soil and, what is probably more important, to increase the rate of transpiration and hence the water requirements of the crop.

Nevertheless statistical studies (2) of weather conditions and wheat yield in western Canada did not demonstrate any pronounced consistent effect of seasonal differences in temperature on the magnitude of the resulting crops, in spite of the fact that retardation of growth owing to deficiency of moisture frequently occurs in this area.

This result might be held to imply that in practice drought damage is largely occasioned by lack of rainfall, and is but little affected by variations in the evaporating power of the air. On the other hand, evaporation itself may be influenced by other meteorological factors to such an extent that temperature records alone fail to indicate adequately the conditions actually operative upon the crop.

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Systematic measurements of evaporation have not long been made at meteorological stations in western Canada. The Bureau of Plant Industry of the United States Department of Agriculture has, however, accumulated such observations at stations in the Great Plains area and elsewhere in the western United States over a period of approximately 20 years. Publication of these (3, 4) has made possible a statistical study of seasonal variations in evaporation from a free-water surface and their correlation with recorded observations of temperature, wind velocity, and vapor pressure, under conditions both similar and dissimilar to those prevailing in southern Saskatchewan and Alberta. In the following sections we shall analyze in this way the data for the months of May, June, July and August provided by two selected stations, one in Montana and the other in New Mexico. The investigation is purely empirical in nature, and was not undertaken with the object of adding to the existing knowledge of the laws of evaporation. Nor will it augment the number of formulas suggested for computing the extent of evaporation from the known values of meteorological factors, 11 of which formulas, being "some of the more important" will be found listed by Raman and Satakopan (5, Table I). Attention will be confined to the above mentioned aspects of variation in evaporation, its correlation with the other weather factors recorded, and the agricultural meteorological implications of the results.

Observational Data

The U.S. Department of Agriculture scheme embraced a considerable number of stations, but the observations at the majority of these did not extend over a sufficient number of years for the present purpose, or suffered from other defects, notably changes in the diameter of the evaporation pan employed, or absence of some wind or vapor pressure records. Two homogeneous series were selected for study, comprising the observations made at Moccasin, Mont. (lat. 47°15′, long. 109°58′ W. approx., alt. 4,228 ft.) and Tucumcari, N. Mex. (lat. 35°30′, long. 103°48′ W. approx., alt. 4,194 ft.). The former station is situated in longitude between Swift Current, Saskatchewan (107°40′ W., approx.) and Medicine Hat, Alberta (110°40′ W. approx.).

Tables I and II give the evaporation, temperature, wind and vapor pressure measurements for the four months May to August inclusive at Moccasin and Tucumcari, as published by Horton (3) for the period prior to 1921, and by Horton and Cole (4) for 1921–32. These authors state that the evaporation pans used were of galvanized iron, 6 ft. in diameter and 24 in. deep. The pans were buried 20 in. in the soil and were kept filled with water to soil surface level, 4 in. below the rim. Instructions required that grass should be kept cut and other obstructions removed from the vicinity so as to permit free wind movement close to the water surface. The anemometer cups were "in general" 20 in. above the rim of the pan, *i.e.*, 24 in. above the water surface.

Mean daily temperatures were obtained by averaging the readings of maximum and minimum thermometers exposed 4ft. above ground in standard

TABLE I

EVAPORATION (E_o) AND MEAN AIR TEMPERATURE (T_a), WIND MILEAGE (W) AND VAPOR PRESSURE (V) BY MONTHS AT MOCCASIN, MONT.

Year	E_o, inches				T_a, degrees F.				W, m.p.h.				V, inches Hg.			
	May	June	July	Aug.	May	June	July	Aug.	May	June	July	Aug.	May	June	July	Aug.
1910	5.61	7.21	8.28	7.38	51	60	68	62	6.8	6.3	5.9	6.0	0.242	0.307	0.330	0.287
11	5.94	5.13	7.31	6.35	48	60	62	58	8.0	6.4	5.9	5.9	.271	.425	.378	.373
12	4.14	6.39	5.97	6.23	52	61	61	62	7.6	5.7	4.3	6.4	.256	.304	.336	.311
13	4.23	4.90	6.37	7.35	49	65	62	65	6.5	5.3	5.0	5.4	.238	.381	.341	.288
14	4.85	4.43	7.45	7.24	51	57	68	62	7.5	6.4	5.5	6.0	.247	.332	.372	.289
15	4.61	4.35	4.89	6.51	49	52	58	66	7.5	6.6	5.1	4.9	.229	.282	.332	.358
16	5.26	5.62	6.89	7.57	45	55	65	62	9.2	7.7	5.6	5.3	.173	.288	.382	.271
17	5.06	6.06	9.58	9.87	46	55	69	63	7.6	7.4	5.9	6.1	.187	.240	.278	.240
18	—	6.76	5.77	5.65	—	63	63	61	—	6.0	4.5	4.9	—	.311	.341	.302
19	6.37	8.57	10.00	8.74	52	64	68	67	8.0	6.9	6.6	6.3	.205	.278	.270	.253
1920	5.36	5.18	6.07	6.58	48	57	66	64	8.8	5.4	4.5	4.8	.191	.293	.365	.299
21	4.14	5.34	6.79	7.58	49	61	64	66	8.3	5.4	5.0	5.3	.223	.346	.341	.261
22	5.49	5.88	6.68	7.80	49	61	63	68	9.8	5.5	4.4	4.9	.199	.355	.336	.354
23	5.44	5.45	6.36	5.58	51	58	66	61	6.7	5.5	4.0	3.5	.221	.324	.452	.360
24	4.90	4.11	6.69	6.81	49	53	63	61	6.7	5.7	4.7	4.9	.205	.301	.315	.266
25	5.94	4.90	8.17	7.57	51	57	66	64	7.8	5.9	5.5	6.6	.211	.323	.336	.270
26	5.09	6.36	7.32	6.31	52	59	66	62	6.9	6.8	5.1	6.4	.230	.280	.358	.324
27	4.30	4.33	7.11	5.56	43	57	63	61	7.5	5.7	4.9	4.6	.197	.318	.408	.346
28	6.88	7.60	7.13	6.05	55	54	63	61	7.0	6.3	4.9	4.6	.218	.270	.402	.276
29	5.29	5.77	9.10	7.85	49	56	68	69	7.5	6.1	5.7	5.7	.204	.294	.277	.294
1930	4.94	7.33	8.28	6.82	49	59	69	67	6.8	6.9	5.3	5.2	.250	.273	.323	.353
31	5.48	6.94	8.74	7.67	52	62	67	66	7.4	6.3	5.8	5.4	.196	.307	.312	.319
32	5.28	5.19	7.69	7.72	53	58	66	65	6.7	5.0	5.2	5.7	.243	.330	.330	.318

TABLE II

EVAPORATION (E_o) AND MEAN AIR TEMPERATURE (T_a), WIND MILEAGE (W) AND VAPOR PRESSURE (V) BY MONTHS AT TUCUMCARI, N. MEX.

Year	E_o, inches				T_a, degrees F.				W, m.p.h.				V, inches Hg.			
	May	June	July	Aug.	May	June	July	Aug.	May	June	July	Aug.	May	June	July	Aug.
1913	10.35	8.72	11.83	10.14	68	71	79	79	7.3	7.2	6.6	5.2	0.292	0.410	0.400	0.419
14	7.29	9.88	8.08	8.96	64	76	76	76	7.4	7.1	3.2	3.9	.361	.470	.551	.487
15	9.91	11.24	10.02	8.41	63	74	76	73	7.1	7.1	6.1	4.3	.241	.342	.458	.441
16	11.32	12.73	11.51	8.88	67	77	80	76	7.8	7.6	5.5	5.5	.187	.302	.390	.471
17	9.48	13.72	13.41	10.02	60	76	82	75	8.2	7.3	6.4	5.4	.216	.302	.416	.450
18	12.63	12.23	12.05	11.57	68	79	80	78	10.0	6.7	6.8	7.0	.205	.377	.460	.418
19	7.78	7.54	9.21	8.56	63	70	75	77	6.7	4.7	4.9	2.9	.456	.543	.573	.561
1920	7.73	9.21	9.54	7.33	64	70	76	69	6.2	5.4	3.8	3.5	.349	.450	.533	.526
21	7.83	6.42	8.38	8.99	66	72	77	77	5.7	3.7	3.3	3.2	.352	.504	.533	.501
22	9.05	9.94	12.18	11.11	65	76	81	82	5.8	5.6	5.5	4.5	.281	.444	.435	.422
23	10.00	10.34	11.31	9.72	66	75	80	77	5.7	6.7	4.9	4.5	.274	.418	.470	.477
24	8.15	12.15	10.11	10.20	62	78	77	78	5.8	5.5	4.9	4.8	.288	.343	.481	.462
25	9.26	12.33	10.09	8.74	67	78	80	75	6.5	7.5	5.1	4.8	.324	.361	.471	.473
26	7.24	9.34	9.51	9.96	63	73	76	77	5.3	4.5	3.9	3.8	.324	.392	.450	.406
27	14.45	11.13	10.37	8.06	72	73	79	75	7.9	7.1	5.1	4.0	.181	.342	.466	.486
28	7.31	10.81	10.81	8.74	65	74	80	75	4.6	5.8	4.9	4.9	.329	.347	.449	.538
29	7.65	10.44	10.86	9.01	62	75	78	78	6.8	6.1	5.2	4.1	.330	.378	.463	.456
1930	10.24	10.66	10.01	9.30	64	76	78	79	7.9	7.0	5.2	4.2	.238	.405	.457	.458
31	7.89	10.27	9.96	8.10	61	76	78	74	5.3	4.8	4.6	3.8	.293	.425	.567	.543
32	9.07	9.47	11.41	10.03	66	73	80	78	6.1	5.0	4.9	5.1	.358	.460	.498	.477

instrument shelters. Temperature, evaporation and anemometer readings were taken at about 7 a.m. and recorded as of the previous day. The vapor pressure measurements given are the averages of the values derived from sling psychrometer readings taken at 7 a.m., 1 p.m. and 5 p.m.

Relation Between Evaporation Recorded and Temperature, Wind and Vapor Pressure Measurements

General variation and covariation

In Fig. 1 is shown for all months and seasons the amount of evaporation plotted against the mean monthly (air) temperature. The evaporation values plotted for June are 31/30 of those given in Tables I and II, thus allowing for the fact that this month comprises only 30 days. It will be seen that a high mean temperature was in general associated with above-average evaporation, and that the relation, as would be expected from physical considerations, is non-linear. Clearly however the amount of evaporation is by no means closely predictable from the mean temperature alone. The curves in Fig. 1 represent the quadratic regression equations

$$E_o = 19.27 - 0.6169T_a + 0.006669T_a^2$$

and

$$E_o = 32.00 - 0.7813T_a + 0.006509T_a^2$$

fitted to the Moccasin and Tucumcari data respectively by the method of Least Squares; but the deviations from the regression line constitute 40.2 and 71.8% respectively of the total variance of evaporation.

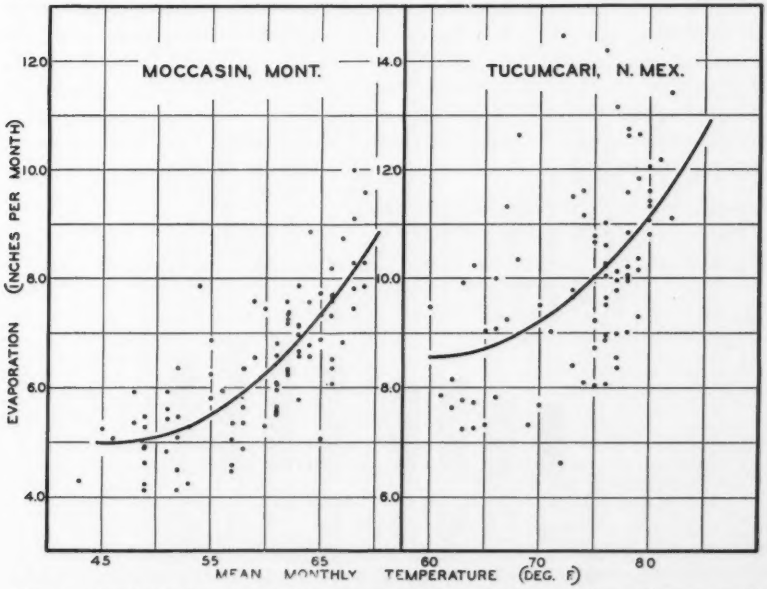

FIG. 1. *Correlation between monthly evaporation from a free-water surface and mean monthly air temperature at Moccasin, Mont., 1910–32, and Tucumcari, N. Mex., 1913–32.*

When the wind mileage and vapor pressure readings were also taken into consideration, however, these correlations were significantly enhanced. It appears that from theoretical considerations the wind effect might be expected to be proportional to the square root of the velocity rather than to the velocity itself; but the experiments of Banerji and Wadia (1) indicate that although the relation is definitely non-linear at low wind velocities, it approaches linearity over the range with which we are here concerned. In these circumstances it was felt that deviations from linearity in the relation between evaporation and average wind velocity were likely to be secondary in importance to errors introduced by the fact that the monthly average does not distinguish between a high wind blowing for a short period and one of low velocity but correspondingly extended duration. The partial regression coefficient of evaporation on mean monthly wind mileage was computed to be 0.36 in. and 0.49 in. per additional m.p.h. at Moccasin and Tucumcari respectively; the corresponding values for evaporation on vapor pressure were -0.95 and -1.06 in. per additional $\frac{1}{10}$ in. of mercury. The multiple correlation coefficient R, between evaporation and the first and second powers of the temperature, wind velocity and vapor pressure now became 0.87 for the Moccasin and 0.94 for the Tucumcari observations

These calculations suffice to indicate that there was in general a correlation between mean air temperature and monthly evaporation from a free-water surface, but that the amount of evaporation at both locations was also significantly affected by differences in vapor pressure and wind mileage. From the agricultural point of view it will be of further interest to consider the annual variation in amount of evaporation during a specified calendar month. This procedure will exclude from the estimates of variance and covariance the effects of any consistent differences between the four months in temperature, wind or vapor pressure, as well as in the temperature of the soil surrounding the evaporation pans.

Annual variation and covariation by months

The upper portion of Fig. 2 shows by columnar heights the average amount of evaporation in each of the four calendar months. Although this was in

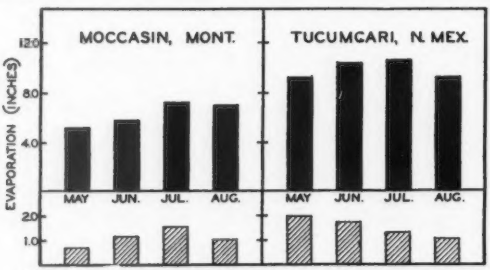

FIG. 2. *Upper portion: Average evaporation by months from a free-water surface at Moccasin, Mont., 1910–32, and Tucumcari, N. Mex., 1913–32. Below: Annual variation (standard deviation) of monthly evaporation.*

all cases higher at Tucumcari than at Moccasin, the seasonal trend is similar at both points, July having the maximum average. Annual variation in the amount of evaporation during a specified month does not, however, follow the same course at the two stations. At Moccasin July was the most variable month, but at Tucumcari annual variation was greatest in May, diminishing in the successive summer months.

Figs. 3 and 4 illustrate in a similar manner the average values and annual variation of air temperature, wind velocity and vapor pressure. They are

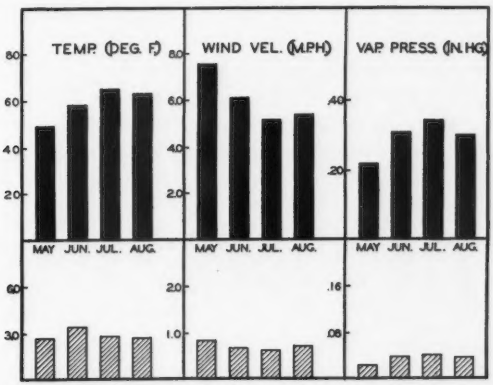

FIG. 3. *Upper portion: Average mean monthly air temperature, wind velocity and vapor pressure at Moccasin, Mont., 1910–32. Below: Annual variation (standard deviation) of mean monthly temperature, wind velocity and vapor pressure.*

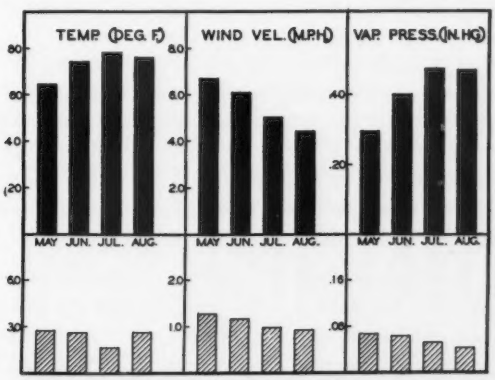

FIG. 4. *Upper portion: Average mean monthly air temperature, wind velocity and vapor pressure at Tucumcari, N. Mex., 1913–32. Below: Annual variation (standard deviation) of mean monthly temperature, wind velocity and vapor pressure.*

of interest in themselves, as indicating the intra- and inter-seasonal variation of these aspects of the weather, but in particular it is to be noted that there are significant average differences between months with respect to both wind

velocity and vapor pressure. These must have operated to reduce the direct correlation between evaporation and temperature in the observations when considered in the preceding section as a whole. At both stations July was the month of maximum average temperature, evaporation and vapor pressure. Average wind velocity was greatest in May, decreasing progressively during the summer.

Evaporation is plotted against mean monthly air temperature for the four months separately in Figs. 5 and 6. The range in temperature of a specified month from year to year is much less than that obtained when all months are thrown together, and the linear regression lines shown seem to fit reasonably adequately the data when subdivided in this way. The computed values of the regression coefficients, specifying the average increase in evaporation per unit increment of mean temperature are:

Moccasin		Tucumcari	
May,	0.114 in. per deg. F.	May,	0.487 in. per deg. F.
June,	0.145 in. per deg. F.	June,	0.511 in. per deg. F.
July,	0.352 in. per deg. F.	July,	0.584 in. per deg. F.
August,	0.193 in. per deg. F.	August,	0.301 in. per deg. F.

The regression coefficients vary somewhat from month to month at both stations. This might perhaps have been in part expected, since the average monthly mean temperature shows a seasonal trend and the effect of a given variation is greater at high than at low temperatures. It must be remembered, however, that these coefficients represent not only the direct result of temperature differences, but also any additional effect due to associated variations in wind or vapor pressure. Upon further examination it was found that the degree of association between inter-annual variations in temperature and wind and temperature and vapor pressure was not the same for all four months. When allowance was made for this situation the partial regression coefficients of evaporation on temperature, eliminating the effects of wind and vapor pressure differences, were found to be:

Moccasin		Tucumcari	
May,	0.166 ± 0.055 in. per deg. F.	May,	0.328 ± 0.054 in. per deg. F.
June,	0.257 ± 0.051 in. per deg. F.	June,	0.256 ± 0.064 in. per deg. F.
July,	0.237 ± 0.044 in. per deg. F.	July,	0.392 ± 0.062 in. per deg. F.
August,	0.171 ± 0.051 in. per deg. F.	August,	0.220 ± 0.045 in. per deg. F.

Differences between the temperature coefficients computed in this way are less pronounced, and those occurring at Moccasin cannot be regarded as statistically significant. At Tucumcari the variation was irregular, and it can be asserted only that the temperature coefficient for August is lower than that for July.

The simple correlation coefficients between evaporation and temperature were found to be:

Moccasin		Tucumcari	
May,	$r = +0.44$	May,	$r = +0.72$
June,	$r = +0.42$	June,	$r = +0.71$
July,	$r = +0.80$	July,	$r = +0.84$
August,	$r = +0.52$	August,	$r = +0.77$

Evidently the recorded temperatures alone would provide a far from reliable prediction of the seasonal variation of evaporation at Moccasin. The degree of correlation is in general higher at Tucumcari, but here also there are some pronounced deviations between actual evaporation and the expectation from the temperature regression. These are in part attributable to variations in wind velocity and vapor pressure. When these variables were also taken into account, the coefficients of multiple correlation, R, between evaporation and temperature, wind velocity and vapor pressure given below were obtained:

Moccasin		Tucumcari	
May,	$R = 0.59$	May,	$R = 0.96$
June,	$R = 0.81$	June,	$R = 0.95$
July,	$R = 0.93$	July,	$R = 0.96$
August,	$R = 0.80$	August,	$R = 0.92$

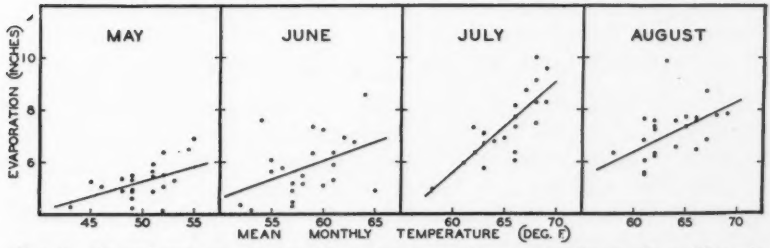

FIG. 5. *Annual variation of mean air temperature and evaporation by months at Moccasin, Mont., 1910–32.*

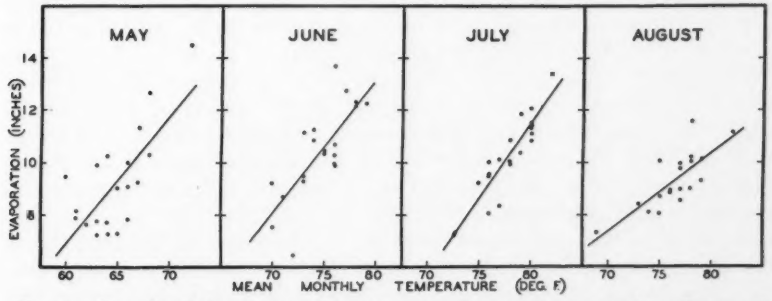

FIG. 6. *Annual variation of mean air temperature and evaporation by months at Tucumcari, N. Mex., 1913–32.*

The general degree of association at Moccasin is still lower than that at Tucumcari.

The limited number of observations results in a high standard error of the partial regression coefficients of evaporation on wind velocity and vapor pressure computed from the annual data for any one month only, since the 22 and 19 degrees of freedom at Moccasin and Tucumcari respectively can hardly be regarded as adequate for the simultaneous determination of three partial regression coefficients. It seemed reasonable to assume however that the effect of a given annual fluctuation in wind velocity or vapor pressure would be much the same for all months. Accordingly, the residual deviations of evaporation, wind velocity and vapor pressure from the temperature regressions for the separate months were combined to provide data for the calculation of an average estimate of these partial regression coefficients, the residual squares and products for June being weighted in the ratio $(31/30)^2$ and 31/30 respectively. The values found in this way were:

Moccasin	Tucumcari	
0.404 ± 0.110,	0.406 ± 0.074	in. per additional mean monthly m.p.h.
-1.13 ± 0.22,	-1.09 ± 0.15	in. per additional mean monthly $\frac{1}{10}$ in. Hg.

The agreement between the values of the coefficients computed from the observations at the two stations (which lie some 800 miles apart) is well within the standard error.

Discussion and Conclusions

Sir Napier Shaw (7) has commented on the uncertainties which may enter into comparisons of the absolute amount of evaporation owing to differences in the instruments employed or their exposure. Topographical differences are doubtless operative, and differences in altitude are also known (6) to have an effect. These, however, would be expected to influence the average amount of evaporation rather than its correlation with other meteorological factors. Nevertheless it seemed desirable to ascertain whether the moderate correlation at Moccasin might be typical of the northern plains area. For this purpose a few additional calculations were made, using the shorter 17-years' series of observations accumulated at Havre, Mont. (lat. 48°40′, long. 109°35′, approx., alt. 2505 ft.). The meteorological quantities showed an average seasonal trend similar to that already illustrated by the Moccasin data. The linear regression of evaporation on temperature was computed to be 0.210, 0.314, 0.508, and 0.330 in. per additional deg. F. mean temperature during May, June, July and August respectively. The corresponding correlation coefficients were +0.75, +0.79, +0.73 and +0.74, *i.e.*, of the same order as those previously computed for Tucumcari. It would appear therefore that the correlation at Moccasin was probably for some reason exceptionally low; but even at the other two stations, a significant proportion of the annual variation in monthly evaporation was unaccounted for by temperature differences alone.

It is not of course contended that evaporation from a free-water surface constitutes a direct measure of the coincident loss of moisture from soil either directly (especially under semi-arid conditions) or by the transpiration of plants. It does, however, provide information respecting the evaporating power of the air, which is the resultant of temperature, wind and humidity conditions; and, since the evaporating power of the air must exercise a controlling influence upon both evaporation from the soil and transpiration, evaporation data of this kind should be of definite utility in supplementing temperature records in agricultural meteorological studies. The relation between evaporation from tanks or pans of water and that from adjacent soil and vegetation would seem to merit investigation.

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STUDIES ON THE INHERITANCE OF AND THE RELATION BETWEEN KERNEL TEXTURE AND PROTEIN CONTENT IN SEVERAL SPRING WHEAT CROSSES[1]

By O. S. Aamodt[2] and J. H. Torrie[3]

Abstract

In crosses of Milturum × Selection I-28-60, F_2 populations grown from different F_1 plants were found to behave in three different ways in regard to the mode of inheritance of kernel texture. In two of these groups vitreous texture was partially dominant and governed by either one or two main factors, while in the third group starchy texture was partially dominant and governed by one main factor pair. Minor modifying factors also influence the mode of inheritance of this character in the above crosses. The heritable nature of kernel texture was also demonstrated, by correlation studies, in the crosses of Reward with Selections I-28-46, I-28-60 and I-28-62.

The inheritance of protein content in Milturum × Selection I-28-60 appeared to be controlled by polymeric factors, the exact nature of which was not determined. The lack of correlation for crude protein content between the material grown at Brooks and that grown at Fallis indicates that the expression of the mode of inheritance of protein content is readily influenced by the environment. In the other crosses studied, protein content was also shown to be inherited.

A strong positive relation was found to exist between vitreous kernel texture and high protein content for the several series of crosses studied.

Introduction

The hard red spring wheats of western Canada are noted the world over for their high milling and baking quality. In order to maintain this reputation, it is essential that the plant breeders of western Canada give the question of quality special consideration in their wheat breeding programs. At the University of Alberta, extensive studies are being carried out on several phases of this general problem. Aamodt and Torrie (2) point out that one of the most serious problems with which wheat breeders have to contend is that of securing an index of baking quality of the different hybrid lines in the early generations of a cross. The practice followed with hybrid material at the University of Alberta is to grow it at Fallis, Alberta, in the gray wooded soil area, where a differentiation in kernel texture is readily secured (1). Aamodt and Torrie (2) have shown that the kernel texture of material grown under Fallis conditions provides an excellent preliminary indication of the baking quality in the early generations of hybrid material.

As a part of the general wheat breeding program at the University of Alberta, the inheritance of kernel texture was studied in several series of

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crosses. The inheritance of protein content and the relation between protein content and kernel texture, under several different environments, were also studied for the same series of crosses.

Materials and Experimental Methods

Genetic studies were made on the inheritance of, and the relation between, kernel texture and protein content in two groups of crosses. The first group consisted of crosses between Reward and Hybrid Selections I-28-46, I-28-60 and I-28-62. The second group consisted of crosses between Milturum 0.321 and Selection I-28-60. The relation between kernel texture and protein content was also studied for 100 white-seeded selections from a cross between Garnet and Selection I-28-46.

The F_1 of Reward crossed with Selections I-28-46, I-28-60 and I-28-62 was grown in the greenhouse during the winter of 1928-1929. The F_2 was grown at Edmonton (black soil) in 1929; the F_3 at Edmonton and Breton (gray soil) in 1930; and the F_4 at Edmonton and Fallis (gray soil) in 1931. The F_1 and F_2 of Milturum × Selection I-28-60 were grown at Edmonton in 1930 and 1931, respectively, and the F_3 at Edmonton (black), Fallis (gray), and Brooks (brown soil), Alberta, in 1932. One hundred white-seeded selections of Garnet × Selection I-28-46 were grown at Fallis in 1931. All the hybrid material was grown in spaced five-foot rows, one foot apart, with approximately 25 seeds per row.

The nature and composition of the black chernozem and gray podsol soils are described by Wyatt, Newton and Mather (25), and the brown soils by Wyatt and Newton (24).

Reward, C.A.N. 1621*, is an early, hard red spring wheat originating from a Marquis × Prelude cross made at the Central Experimental Farm, Ottawa. It is characterized by vitreous texture, high protein content and excellent milling and baking qualities. Garnet, C.A.N. 1316, is an eariy, hard red spring wheat originating from a Preston A × Riga M cross made at the Central Experimental Farm, Ottawa. It is characterized by vitreous texture, below-average protein content and fair to poor milling and baking qualities. Milturum 0.321, C.A.N. 1415, is a late, soft red spring wheat obtained from Dr. Talanov of the West Siberian Experimental Station, in 1928. It is characterized by starchy texture, low protein content and poor milling and baking qualities. Selections I-28-46, I-28-60 and I-28-62 are hard red spring wheats originating from a cross of Marquillo × Marquis-Kanred. They were introduced from the University of Minnesota, St. Paul, in 1928, and were later selected at the University of Alberta, Edmonton. They are all relatively high in protein content and possess good milling and baking qualities. At Fallis, on the gray soil, all three vary somewhat in kernel texture.

The protein content was calculated as total nitrogen × 5.7 and corrected to a 13.5% moisture basis. The material was classified for kernel texture on a scale ranging from 1 to 10; 1 represents completely starchy and 10 completely vitreous.

* *C.A.N. = Canadian Accession Number.*

The significance of the correlation coefficients, reported herein, was judged by the method suggested by Fisher (14). A correlation was considered significant if P, the probability of exceeding the observed value through random sampling, was less than 0.05. In all instances throughout this paper the standard error is used.

Literature Review

In the early literature dealing with kernel texture there is a considerable amount of discussion on the influence of environment. Very little consideration, save in a few isolated cases, is given to the hereditary nature of kernel texture. However, more recently, considerable literature has accumulated on the mode of inheritance of this character. Owing to the importance of kernel texture in the breeding program at the University of Alberta, and in this paper, the literature on the subject is reviewed in considerable detail.

Bolley, 1905 (6) states that "yellow-berry" in wheat is determined by climatic conditions and that it is not inherited. Lyon and Kezer, 1905 (21) report that yellow-berry is produced primarily by over-ripening and the length of time exposed to the weather.

Biffen, 1905 (4) was probably the first to show clearly the heritable nature of kernel texture. In crosses between a hard Polish wheat and a soft Rivet wheat, he obtained in the F_2 a ratio of three hard to one soft for kernel texture. In a later paper, 1908 (5), in crosses between Rough Chaff (soft) and Fife (hard), he reported that the hard translucent endosperm of Fife behaved as a simple dominant to the soft opaque endosperm of Rough Chaff.

Roberts and Freeman (23), in 1908, stated that both environment and heredity play a role in determining yellow-berry. Headden, 1915 (18), studied the effect of different commercial fertilizers on yellow-berry. He concluded that the nitrogen/potassium ratio greatly influenced the production of yellow-berry; a high proportion of nitrogen favored the production of flinty kernels, while a high proportion of potassium favored yellow-berry.

Howard and Howard, 1912 (19), obtained a monogenic ratio, with an intermediate condition in the F_1, for kernel texture from crosses between soft and hard translucent wheats. Freeman, 1918 (15), studied the inheritance of kernel texture in crosses between a white macaroni wheat and (*a*) a soft red bread wheat and (*b*) Sonora 35, a soft white wheat. He proposed that two independent factor pairs, cumulative in nature, conditioned the inheritance of kernel texture in these crosses. He states that in his material there were two types of soft texture in grain; "true softness", which is only slightly affected by environmental conditions, and "yellow-berry", which is very sensitive to environmental conditions. He believed that the genetic factors governing the expression of these two types are different.

Roberts, 1919 (22), studied yellow-berry in winter wheat, from which he concluded that the operation of environmental causes of yellow-berry overshadows any differences which may be due to heredity. However, he was

able to isolate pure strains of wheat which were freer from yellow-berry than others grown under identical conditions. Leith, 1919 (20), reports that the yellow-berry condition found in wheat is not heritable. However, he found considerable differences in pure lines of wheat in their tendency to produce yellow-berry. He cites the work of LeClerc and Leavitt, who also conclude that yellow-berry is caused by environmental conditions.

Bryan and Pressley, 1920 (7), crossed Turkey Red and Sonora and found the kernel texture of the F_1 to be intermediate between that of the parents. The F_2 data indicated a one-factor differential governing the inheritance of kernel texture. Harrington, 1922 (16), from the results of a series of crosses between spring wheats, concluded that the inheritance of kernel texture is governed by several factors. Hayes, 1923 (17), in a study of Marquis × Preston, obtained a correlation of $+0.407 \pm 0.059$ between the F_4 and F_5 for kernel texture. He states that, while environment plays a major role in determining kernel texture, nevertheless heredity is of importance. Engledow and Hutchinson, 1926 (13), made crosses between *T. turgidum* (soft) and *T. durum* (hard) and obtained in the F_1 completely vitreous kernels, indicating a complete dominance of the durum endosperm. They suggest that two factors govern the inheritance of kernel texture, which separately give a durum-like endosperm and jointly a true durum endosperm. Clark, Florell and Hooker, 1928 (10), studied kernel texture in Hard Federation (vitreous) × Propo (soft) wheat crosses. The F_2 and F_3 were classified into five groups for texture, as judged by the outward appearance of the kernels. The F_2 distribution approached a normal curve, with a preponderance of those possessing vitreous texture. In the F_3, they obtained indication of transgressive segregation and also true breeding intermediate lines. They concluded that kernel texture is strongly inherited and suggested that several factor pairs are involved. Bryan and Pressley, 1933 (8), crossed Baart, a vitreous wheat, and Sonora 103, a soft wheat. The F_1 plants were as soft as Sonora 103, showing a dominance of soft texture. The F_2, F_3 and F_4 showed segregation for this character and indicated a single main factor with numerous modifying factors. Aamodt, Torrie and Wilson, 1935 (3), in crosses between Reward and Milturum, report a dominance of starchy texture, and that the inheritance of kernel texture is governed by polymeric factors.

Experimental Results

Reward crosses

The F_3 of Reward crossed with each of the three Selections I-28-46, I-28-60 and I-28-62 was grown at Breton in 1930. Two hundred F_3 plants, selected at random from each cross, were grown in the F_4 at Fallis in 1931. The kernel texture index was determined for each of the F_3 plants and the average of each F_4 line. The data in Table I give the distribution, mean, and standard deviation of the hybrids and parents, for kernel texture index, for both the F_3 and F_4. The variation in kernel texture of the Reward crosses grown at Edmonton, in both 1930 and 1931 was slight. It will be noted, however,

that in the case of Selection I-28-60 × Milturum a satisfactory differentiation in kernel texture was obtained in the material grown at Edmonton, in 1931 (Table III). These results can be explained on the basis that Milturum produces a starchy kernel in most environments, whereas Selections I-28-46,

TABLE I

FREQUENCY DISTRIBUTION FOR KERNEL TEXTURE OF PARENTAL LINES AND CROSSES GROWN IN THE GRAY SOIL AREA AT BRETON IN THE F_3, AND AT FALLIS IN THE F_4

Parent or Cross		Texture classes										Total number	Mean	Standard deviation
		1	2	3	4	5	6	7	8	9	10			
Reward	P_3	—	—	—	—	—	—	—	-	17	18	35	9.5 ± 0.10	0.57
	P_4	—	—	—	—	—	—	1	14	20	—	35	8.5 ± 0.10	0.60
I-28-46	P_3	—	1	1	3	1	4	1	2	2	—	15	5.8 ± 0.55	2.07
	P_4	—	—	1	3	1	4	4	2	—	—	15	5.9 ± 0.40	1.49
I-28-60	P_3	—	—	—	—	—	—	2	4	9	—	15	8.5 ± 0.18	0.68
	P_4	—	—	—	—	1	1	4	6	3	—	15	7.6 ± 0.29	1.08
I-28-62	P_3	—	1	1	—	1	3	2	5	2	—	15	6.7 ± 0.53	1.98
	P_4	—	—	—	—	1	2	6	4	2	—	15	7.3 ± 0.28	1.04
Reward × I-28-46	F_3	34	16	24	10	6	8	6	12	55	27	198	5.8 ± 0.24	3.44
	F_4	—	—	9	14	23	28	25	43	54	2	198	7.0 ± 0.13	1.84
Reward × I-28-60	F_3	—	1	7	10	11	14	9	16	66	66	200	8.3 ± 0.15	2.07
	F_4	—	—	2	4	6	14	23	30	93	28	200	8.2 ± 0.11	1.50
Reward × I-28-62	F_3	11	9	18	10	13	17	12	3	65	42	200	7.0 ± 0.21	2.92
	F_4	—	—	1	11	16	24	29	38	59	22	200	7.7 ± 0.12	1.72

I-28-60 and I-28-62 require a special environment, such as that in the gray soil area, to bring out inherent differences in kernel texture. Reward is shown to be homogeneous for vitreous texture, but the great variability in kernel texture of the three Selections indicates that they are all probably heterogeneous for kernel texture. The influence of heredity on kernel texture is indicated by the close relation existing between the mean texture indices of the three Selections and those of the corresponding hybrid populations. The mean texture indices of the three Selections, for the P_3 and P_4, are: I-28-60, 8.5 and 7.6; I-28-62, 6.7 and 7.3; I-28-46, 5.8 and 5.9; while those for the corresponding crosses with Reward, in the F_3 and F_4, are: Reward × I-28-60, 8.3 and 8.2; Reward × I-28-62, 7.0 and 7.7; Reward × I-28-46, 5.8 and 7.0, respectively. These results show a direct relation between the texture indices of the parents and of the resulting hybrids, indicating that the Selections differ genetically for factors governing kernel texture. The three series of crosses show considerable variability for kernel texture. However, owing to the heterogeneous nature of the three selections used as parents, and the limited amount of material grown, a detailed genetical analysis was not possible.

Correlation coefficients were calculated between the kernel texture index of the F_3 plants and the corresponding F_4 lines, for all three series of crosses, in order to show further the heritable nature of kernel texture.

The correlations shown by the data in Table II clearly indicate that, in the crosses studied, kernel texture is a heritable character governed by definite genetic factors.

TABLE II

CORRELATIONS BETWEEN THE KERNEL TEXTURE OF THE F_3 AND F_4 PLANTS

Cross	Number	r
Reward × I-28-46	198	+0.669**
Reward × I-28-60	200	+0.482**
Reward × I-28-62	200	+0.638**

** *P value exceeds the 1% point.*

Milturum crosses

Detailed genetic studies were made on the inheritance of kernel texture in the F_2 and F_3 of crosses between Selection I-28-60 and Milturum. The F_2 was grown in 1931 at Edmonton, where, owing to a moist season, a fair differentiation in kernel texture was obtained. The average texture for each F_3 line was determined at Edmonton, Fallis and Brooks in 1932. A good differential reaction for kernel texture was obtained only in the lines grown at Fallis; at Edmonton and Brooks insufficient variation occurred in kernel texture of the lines grown to make a genetical study possible.

The distribution of kernel texture within populations produced from different F_1 plants showed considerable variation both in the F_2 and F_3. The kernel texture of the grain from each plant in the F_2 populations was determined. The average for each line was calculated, and on the basis of this the populations were classified into groups. Those with a low average kernel texture were designated as starchy; those with a high average kernel texture were called vitreous and the remainder were classed as semi-vitreous. The data obtained in the F_3 were similarly treated, the progeny of each F_2

TABLE III

FREQUENCY DISTRIBUTION FOR KERNEL TEXTURE INDEX OF THE THREE TEXTURE GROUPS FROM SELECTION I-28-60 × MILTURUM AND THE PARENTAL LINES

Texture groups	Texture classes										Total number	Mean	Standard deviation
	1	2	3	4	5	6	7	8	9	10			
F_3 Edmonton, 1931													
Starchy	2	10	16	16	12	5	9	6	4	—	80	4.6 ± 0.23	2.09
Semi-vitreous	1	1	2	2	4	10	15	16	15	7	73	7.3 ± 0.22	1.91
Vitreous	1	—	—	—	1	6	21	47	34	16	126	8.2 ± 0.11	1.27
F_3 Fallis, 1932													
Starchy	14	46	39	34	13	17	21	8	2	—	194	3.9 ± 0.15	2.03
Semi-vitreous	4	10	12	14	13	26	34	29	24	2	168	6.2 ± 0.17	2.22
Vitreous	1	—	3	5	4	15	36	47	30	10	151	7.6 ± 0.12	1.47
I-28-60	—	—	—	—	1	1	2	2	4	1	11	7.9 ± 0.46	1.44
Milturum	11	—	—	—	—	—	—	—	—	—	11	1.0 ± 0.00	—

plant being regarded as a separate line. The kernel texture distributions of the lines in each group were then summed. These summations, together with their means and standard deviations, for the hybrid material grown at Edmonton in 1931 and at Fallis in 1932, and for the parental lines grown at Fallis in 1932, are given in Table III.

The data in Table III show that the distribution and the mean of the texture index of the three groups, for both the F_2 grown at Edmonton and the F_3 grown at Fallis, differ considerably. The data in Table IV show that the differences between these groups are significant in both the F_2 and F_3 and between parental lines.

TABLE IV

SIGNIFICANCE OF THE DIFFERENCE IN TEXTURE INDEX OF THE THREE TEXTURE GROUPS FROM SELECTION I-28-60 × MILTURUM AND THE PARENTAL LINES

Texture or parental groups	F_2 Edmonton		F_3 Fallis	
	Difference	Diff./S.E.	Difference	Diff./S.E.
Starchy and semi-vitreous	2.7 ± 0.32	8.4	2.3 ± 0.23	10.1
Starchy and vitreous	3.5 ± 0.25	14.2	3.7 ± 0.19	19.5
Semi-vitreous and vitreous	0.9 ± 0.25	3.4	1.4 ± 0.21	6.6
I-28-60 and Milturum	—	—	6.9 ± 0.44	15.7

Statistical significance is taken at 2 × S.E.

In the F_2 a break in the distribution occurs at class 6, while in the F_3 the break occurs at class 5. Taking these classes as arbitrary dividing points in both the F_2 and F_3, excellent fits to the expected ratios are obtained in every case, with the exception of the F_3 of the semi-vitreous group where a P value of 0.05 was obtained. The results are shown in Table V.

TABLE V

THE BREEDING BEHAVIOR OF PLANTS IN THE THREE TEXTURE GROUPS FROM SELECTION I-28-60 × MILTURUM

Generation and texture group	Class	Observed	Calculated	χ^2	P lies between
F_2 starchy	starchy	61	60	0.07	0.80 and 0.70
	vitreous	19	20		
F_2 semi-vitreous	starchy	20	18.3	0.21	0.70 and 0.50
	vitreous	53	54.7		
F_2 vitreous	starchy	7	7.9	0.11	0.80 and 0.70
	vitreous	119	118.1		
F_3 starchy	starchy	146	145.5	0.01	0.95 and 0.90
	vitreous	48	48.5		
F_3 semi-vitreous	starchy	53	42	3.84	0.05
	vitreous	115	126		
F_3 vitreous	starchy	12	9.4	0.77	0.50 and 0.30
	vitreous	139	141.6		

The data indicate that one or both of the parents are heterogeneous for kernel texture. Phenotypically Milturum is homogeneous for starchy endosperm. In 1932 all the parental lines of Milturum when grown at Fallis had a texture index of 1, while Selection I-28-60 had a range from 5 to 10, which indicates that Selection I-28-60 may be heterogeneous for kernel texture. One very interesting aspect of this study is that in certain F_2 populations starchy texture is partially dominant by one main factor and in others vitreous texture is partially dominant by either one or two main factors. In the F_3, grown at Brooks and Edmonton, there was not sufficient variation in kernel texture to allow a detailed study of this character. However, it was noticed that invariably the starchy texture groups had a much higher proportion of starchy plants at both Edmonton and Brooks than either the semi-vitreous or vitreous texture groups. A review of the literature shows that in some cases vitreous texture had been found dominant and in other cases starchy or soft texture was dominant. The only possible explanation for the behavior of the material in these crosses is that Selection I-28-60 is heterogeneous for factors governing kernel texture. In the vitreous texture group there must be two main factors governing texture, and in the semi-vitreous group one main factor, and in both cases a partial dominance of vitreous texture, while in the starchy texture group, starchy texture is partially dominant by a single factor.

The inheritance of kernel texture is also probably influenced by a number of modifying factors as well as the main factors indicated above. Correlation coefficients were calculated between the kernel textures of the three texture groups, grown in the F_2 at Edmonton and in the F_3 at Fallis. These data are shown in Table VI.

TABLE VI

INTER-GENERATION CORRELATIONS FOR THE THREE TEXTURE GROUPS OF PLANTS FROM SELECTION I-28-60 × MILTURUM

Variables correlated		Total number	r
Vitreous group	F_2 and F_3	123	+0.404**
Semi-vitreous group	F_2 and F_3	63	+0.706**
Starchy group	F_2 and F_3	80	+0.885**

** *P value exceeds the 1% point.*

Protein Content

Literature review

Protein content is one of the most important indications of quality in wheat. With the exception of the papers of Clark and his associates, there is a dearth of literature on the mode of inheritance of protein content in wheat.

Biffen, 1908 (5), working with crosses between a Polish wheat high in nitrogen content and Rivet wheat, low in nitrogen content, obtained segregation in the F_2 for this character. He states that high nitrogen content was

associated with hard translucent kernels. Clark, 1926 (9), obtained transgressive segregation for protein content beyond the upper extremes of both parents in the F_4 of reciprocal crosses of Kota × Hard Federation. Clark and Hooker, 1926 (11), with reciprocal crosses of Marquis and Hard Federation, found that the protein content for the F_2 and F_3 of the hybrids was intermediate between that of the parents, and that there was no indication of transgressive segregation. They state that protein content is inherited in intermediate degrees between the two parents by means of multiple factors. Correlations between the protein content of the F_2 and the F_3 were not significant, which illustrates how readily this character is influenced by the environment. Clark, Florell and Hooker, 1928 (10), in a series of crosses between Bobs, Hard Federation and Propo, found a tendency for low protein content to be dominant. Correlations between the F_2 and F_3 were not significant. Clark and Quisenberry, 1929 (12), found that the F_2 and the F_3 of a Marquis × Kota cross were intermediate between, and more variable than, either parent. Several F_3 strains had a lower protein content than Marquis the lower parent, indicating transgressive segregation. Correlations between the F_2 and F_3 protein content were highly significant.

Experimental results

Reward crosses. In the crosses of Reward with Selections I-28-46, I-28-60 and I-28-62, the protein content was determined on 20 lines of each cross grown in the F_3 at Edmonton and Breton, and in the F_4 at Edmonton and Fallis, but not on the F_3 of Reward × Selection I-28-46 at Edmonton. The F_3 lines chosen for the protein determinations were selected on the basis of their F_2 behavior for texture. Ten lines of each cross with a starchy texture and ten with a vitreous texture were used.

The correlation coefficients obtained for the various comparisons of protein content in the crosses of Reward with Selections I-28-46, I-28-60 and I-28-62 are given in Table VII. In each of the correlations a population of 20 was used.

TABLE VII

INTER-GENERATION AND INTER-STATION CORRELATIONS FOR PROTEIN CONTENT OF WHEAT

Crosses and association	r
Reward × I-28-46, F_3 Breton and F_4 Fallis	0.510*
Reward × I-28-60, F_3 Breton and F_4 Fallis	0.848**
Reward × I-28-62, F_3 Breton and F_4 Fallis	0.766**
Reward × I-28-60, F_3 and F_4 Edmonton	0.375
Reward × I-28-62, F_3 and F_4 Edmonton	0.441*
Reward × I-28-46, F_3 Edmonton and Breton	0.510*
Reward × I-28-60, F_3 Edmonton and Breton	0.257
Reward × I-28-60, F_4 Edmonton and Fallis	0.701**
Reward × I-28-62, F_3 Edmonton and Breton	0.631**
Reward × I-28-62, F_4 Edmonton and Fallis	0.620**

* *P value exceeds the 5% point.* ** *P value exceeds the 1% point.*

TABLE VIII

FREQUENCY DISTRIBUTIONS FOR PROTEIN CONTENT OF WHEAT GROWN AT FALLIS, EDMONTON AND BROOKS, IN 1932

	Classes for protein content in per cent																	Number	Mean	Standard deviation
	9.8	10.3	10.8	11.3	11.8	12.3	12.8	13.3	13.8	14.3	14.8	15.3	15.8	16.3	16.8	17.3	17.8			
Fallis (gray soil)																				
Milturum	—	—	—	—	—	1	2	3	—	1	2	—	—	—	—	—	—	9	13.5±0.47	1.41
Selection I-28-60	—	—	—	—	—	—	—	1	—	3	3	2	—	—	—	—	—	9	14.6±0.19	0.57
F_2	3	12	15	12	19	11	15	11	14	11	5	4	—	—	—	—	—	132	12.4±0.13	1.45
Edmonton (black soil)																				
Milturum	—	—	—	—	—	1	—	1	1	—	—	—	—	—	—	—	—	3	13.1	—
Selection I-28-60	—	—	—	—	—	—	—	—	—	—	—	1	1	1	—	—	—	3	15.8	—
F_2	—	—	—	—	—	—	—	—	—	8	5	11	4	7	2	5	—	42	15.4±0.15	0.96
Brooks (brown soil)																				
Milturum	—	—	—	—	—	—	—	—	—	—	—	1	—	1	1	—	—	3	16.2	—
Selection I-28-60	—	—	—	—	—	—	—	—	—	—	—	—	1	2	—	—	—	3	16.2	—
F_2	—	—	—	—	—	—	—	—	—	—	—	4	6	13	15	3	1	42	16.4±0.09	0.57

For all but two comparisons, the correlations are significant. The correlations of Reward × Selection I-28-60 both between the F_3 and F_4 grown at Edmonton and between the F_3 grown at Edmonton and Breton were positive but not significant. These data clearly demonstrate that protein content is a heritable character.

Milturum crosses. The protein content was determined on 42 F_3 lines from Milturum × Selection I-28-60 crosses, grown at Brooks and Edmonton, and 132 F_3 lines grown at Fallis, in 1932. In Table VIII the data give the distribution, mean and standard deviation for the F_3 lines and parents of Milturum × Selection I-28-60 grown at the three different stations.

The protein determinations of the grain from parental lines, grown at Edmonton and Brooks, were too few in number for statistical analysis of the data. The data may be used, however, to show the general trend in protein content of the parental lines. When grown at Edmonton, there was a difference of 2.7% in the average protein content of the parents, indicating an inherent tendency for Selection I-28-60 to be higher in protein content than Milturum. When grown at Brooks, however, both parents had an average protein content of 16.2%. The fact that no difference occurred in the protein content of the parental lines grown at Brooks indicates that the dry soils, and consequent low yields obtained in this area, afford a poor differentiation for the study of inherent differences in protein content. When grown at Fallis, a difference of $1.1 \pm 0.51\%$ was obtained between Selection I-28-60 and Milturum, which indicates that under Fallis conditions Selection I-28-60 has the same inherent capacity for the production of protein as Milturum. The F_3 grown at Fallis showed considerable transgressive segregation for protein content beyond that of the low parent, Milturum. No lines with a higher protein content than Selection I-28-60 were obtained. In the F_3 lines grown at both Edmonton and Brooks indications of transgressive segregation were obtained. However, owing to the small number of parental lines studied, these indications cannot be considered significant.

The average protein content of the F_3 lines grown at Fallis, Edmonton and Brooks of 12.4 ± 0.13, 15.4 ± 0.15, and 16.4 ± 0.09, respectively, are all significantly different. This clearly illustrates the great influence which different environmental conditions, such as climate and soil type, can have upon the protein content of the same material. The results show that the genetic factors governing this character can best be determined in an environment which gives a different phenotypic expression to each of the genotypic combinations. The distribution in Table VIII indicates that polymeric factors govern the mode of inheritance of protein content in this cross.

The data in Table IX give the correlation coefficients for protein content between the F_3 lines grown at Edmonton, Brooks and Fallis. The correlation between Edmonton and Fallis is highly significant, but between Edmonton and Brooks, and Brooks and Fallis, the correlations are not significant. These results demonstrate further that at Brooks, on the dry brown soils, there is little differentiation between the lines because the environment

dominates to such an extent that the protein content of all varieties tends to reach a common maximum. At Edmonton on the black soils the influence of environment is less than at Brooks, and certain lines show greater variations in protein content from the average. At Fallis, on the gray soil, heredity becomes a more dominating factor in determining the phenotype, and there is a consistent wide difference in protein content between different lines. Only those lines which have a strong hereditary tendency to produce high protein will be above the general average.

TABLE IX

INTER-STATION CORRELATIONS FOR PROTEIN CONTENT OF MILTURUM × SELECTION I-28-60 F_3 LINES

Variables correlated	Total number	r
Edmonton and Fallis	42	+0.509**
Edmonton and Brooks	42	+0.289
Brooks and Fallis	42	−0.160

** *P value exceeds the 1% point.*

The Relation of Kernel Texture to Protein Content

Literature review

The literature on the relation between kernel texture and protein content in varietal studies reviewed by Aamodt and Torrie (2) shows that certain workers reported associations between kernel texture and protein content, while other workers found no association. The relation between these two characters in genetic studies has been studied by Clark and his co-workers. Clark, 1926 (9), with Hard Federation × Propo crosses grown at Davis, California, found a high positive correlation between texture and protein content in the F_3. He states, however, that high protein content is not necessarily associated with hard texture, which is indicated by both the lack of significant correlations between the F_2 and the F_3 for protein content and the partial dominance of low protein content and vitreous texture in the F_2. Clark, Florell and Hooker, 1928 (10), in crosses between Bobs, Hard Federation and Propo wheats, report that there is a slight tendency toward increased protein content with the increase in vitreousness of the kernel texture.

Experimental results

The relations existing between the two characters, kernel texture and protein content, were studied in three groups of crosses. These are: Reward crossed with Selections I-28-46, I-28-60 and I-28-62, in both the F_3 grown at Breton and the F_4 grown at Fallis, the F_3 of Milturum × Selection I-28-60, grown at both Edmonton and Fallis, and 100 white seeded F_4 lines of Garnet × Selection I-28-46 grown at Fallis.

Reward crosses. The data in Table X show that in every case the difference in protein content between the vitreous and starchy textured lines is statistically significant, with the one exception of the F_3 of Reward × Selection I-28-62, grown at Edmonton. The difference between the vitreous and

TABLE X

FREQUENCY DISTRIBUTION FOR PROTEIN CONTENT, OF THE VITREOUS AND STARCHY TEXTURE F_3 AND F_4 LINES GROWN ON BOTH THE GRAY AND BLACK SOILS

Material		Classes for protein content in per cent													Mean	Standard deviation
		8.5	9.5	10.5	11.5	12.5	13.5	14.5	15.5	16.5	17.5	18.5	19.5	20.5		
Reward × I-28-46 F_3 Breton	V[1]	–	–	2	2	3	3	–	–	–	–	–	–	–	12.2±0.37	1.10
	S[2]	–	5	4	1	–	–	–	–	–	–	–	–	–	10.1±0.22	0.66
F_4 Fallis	V	–	–	–	5	4	1	–	–	–	–	–	–	–	12.1±0.22	0.66
	S	–	–	3	7	–	–	–	–	–	–	–	–	–	11.2±0.15	0.46
F_4 Edmonton	V	–	–	–	–	–	–	–	–	5	5	–	–	–	17.0±0.21	0.63
	S	–	–	–	–	–	–	3	4	3	–	–	–	–	15.5±0.26	0.77
Reward × I-28-60 F_3 Breton	V	–	–	–	1	–	2	2	3	2	–	–	–	–	14.7±0.49	1.48
	S	–	–	2	5	1	2	–	–	–	–	–	–	–	11.8±0.33	1.00
F_3 Edmonton	V	–	–	–	–	–	–	–	–	–	5	5	–	–	18.0±0.21	0.63
	S	–	–	–	–	–	–	–	–	3	6	1	–	–	17.3±0.20	0.60
F_4 Fallis	V		–	–	–	1	6	3	–	–	–	–	–	–	13.7±0.20	0.60
	S	–	–	4	3	2	1	–	–	–	–	–	–	–	11.5±0.33	1.00
F_4 Edmonton	V	–	–	–	–	–	–	–	–	5	4	1	–	–	17.1±0.22	0.66
	S	–	–	–	–	–	–	–	6	4	–	–	–	–	15.9±0.19	0.58
Reward × I-28-62 F_3 Breton	V	–	–	–	–	1	4	3	2	–	–	–	–	–	14.1±0.32	0.97
	S	1	1	6	2	–	–	–	–	–	–	–	–	–	10.4±0.28	0.84
F_3 Edmonton	V	–	–	–	–	–	–	–	–	–	5	4	1	–	18.1±0.22	0.66
	S	–	–	–	–	–	–	–	–	1	7	2	–	–	17.6±0.18	0.54
F_4 Fallis	V	–	–	–	1	4	2	1	1	1	–	–	–	–	13.5±0.49	1.48
	S	–	2	1	5	2	–	–	–	–	–	–	–	–	10.9±0.43	1.28
F_4 Edmonton	V	–	–	–	–	–	–	–	–	3	4	1	1	1	17.8±0.43	1.27
	S	–	–	–	–	–	–	1	3	3	3	–	–	–	16.3±0.33	0.98

[1] *V = Vitreous.* [2] *S = Starchy.*

starchy textured lines, in this case, is slightly under twice the standard error of the difference. It is interesting to note that, in spite of the fact that the difference in kernel texture of the vitreous and starchy textured lines was masked by the environment, when grown at Edmonton, the difference in protein content of the two groups of lines was statistically significant for all series of crosses in both the F_2 and F_3, with the one exception noted above. This clearly demonstrates the necessity of growing the material in a differential environment, in order to show differences in quality by kernel texture. These results show the close relation which exists between protein content and the kernel texture, as determined on material grown in the gray soil area. The great influence of environment on protein content is illustrated by the differences in protein content, ranging from three to six per cent, between samples of the same material grown on the two different soil types.

The mean protein content and kernel texture of the parents used in the above crosses are shown by the data in Table XI. Since the protein content

TABLE XI

MEAN PROTEIN CONTENT AND KERNEL TEXTURE OF PARENTAL LINES IN 1931

Parent	P_3				P_4			
	Breton		Edmonton		Fallis		Edmonton	
	Protein, %	Kernel texture	Protein, %	Kernel texture	Protein, %	Kernel texture	Protein, %	Kernel texture
Reward	12.5	9	17.8	10	13.1	9	17.7	10
I-28-46	11.2	7	15.2	10	11.4	7	—	10
I-28-60	11.7	8	16.3	10	11.0	7	15.4	10
I-28-62	10.7	7	15.9	10	11.0	6	18.0	10

was determined on only three samples of each parent, for any one mean, standard deviations were not calculated to show the variability. The variation in protein percentage, in all cases, was small, rarely exceeding one per cent.

The data in Table XII give the correlation coefficients between kernel texture and protein content of Reward crossed with Selections I-28-46, I-28-60 and I-28-62 for both the F_3 and F_4 generations. The correlations are based on populations of 20 in each case.

These correlations are all highly significant and further indicate that, when the material is grown on the gray soils, there exists a very strong positive correlation between kernel texture and protein content.

TABLE XII

CORRELATIONS BETWEEN KERNEL TEXTURE AND PROTEIN CONTENT

Cross and station	r
Reward × I-28-46, F_3 Breton	+0.841**
Reward × I-28-46, F_4 Fallis	+0.548*
Reward × I-28-60, F_3 Breton	+0.786**
Reward × I-28-60, F_4 Fallis	+0.849**
Reward × I-28-62, F_3 Breton	+0.921**
Reward × I-28-62, F_4 Fallis	+0.899**

* *P value exceeds the 5% point.*
** *P value exceeds the 1% point.*

Milturum crosses. It was previously shown that, in the crosses of Milturum with Selection I-28-60, there were three distinct groups of F_2 populations for the mode of inheritance of kernel texture. In Table XIII will be found the correlation coefficients for each of the three texture groups grown at Fallis and for the total population, when grown at both Edmonton and Fallis, between kernel texture and protein content.

TABLE XIII

CORRELATIONS BETWEEN PROTEIN CONTENT AND KERNEL TEXTURE FOR THE F_2 OF MILTURUM × SELECTION I-28-60

Variables correlated	No. of samples	r
Texture and protein, vitreous texture group at Fallis	30	+0.668*
Texture and protein, semi-vitreous texture group at Fallis	39	+0.473*
Texture and protein, starchy group at Fallis	63	+0.551*
Texture and protein, total population at Fallis	132	+0.503*
Texture and protein, total population at Edmonton	42	−0.027

** P value exceeds the 1% point.*

The data in Table XIII show that the correlations between these two characters are very significant for the hybrids grown at Fallis, but not for those grown at Edmonton. The environmental conditions at Edmonton during the growing season of 1932 were not favorable for securing a good differentiation in kernel texture. As a result very little variation was obtained for the kernel texture of the lines grown, and this may account, to a large degree, for the lack of a significant correlation between protein content and kernel texture of the hybrids grown at Edmonton in the year 1932. It was thought that since kernel texture of the hybrids grown at Fallis was so strongly correlated with protein content, there would be a wide difference in the average protein content of the three texture groups. This was not found to be the case. The average protein content was 12.2 ± 0.25, 12.5 ± 0.40 and 12.7 ± 0.37 for the starchy, semi-vitreous and vitreous textured groups, respectively. The differences between the average protein contents of the three groups are not significant. The reason for this apparent paradox is shown by the data in Table XIV.

TABLE XIV

RELATIONS EXISTING BETWEEN THE TEXTURE INDEX AND PROTEIN CONTENT, FOR THE F_3 OF MILTURUM × I-28-60, GROWN AT FALLIS IN 1932

Range of texture index	Average percentage of protein		
	Starchy texture group	Semi-vitreous texture group	Vitreous texture group
1–5	11.9±0.33	11.9±0.50	11.0±0.57
6–10	13.3±0.83	12.9±0.56	13.2±0.51
Ave. 1–10	12.2±0.25	12.5±0.40	12.7±0.37

The data in Table XIV show that the more starchy textured lines of the starchy texture group had a higher protein content than the starchy textured lines of the

vitreous texture group; the same relation also held for the more vitreous textured lines of these groups. These differences were sufficient to balance the expected significant differences between the groups in average protein content.

Garnet crosses. A number of F_4 white-seeded segregates from a Garnet × Selection I-28-46 cross were grown at Fallis in 1931. The selections were classified into four categories for kernel texture. These categories were: vitreous, semi-vitreous, semi-starchy and starchy. The protein content was determined on 100 individual plants, 25 from each category. The mean protein content for each of the four categories is shown by the data in Table XV.

TABLE XV

THE MEAN PROTEIN CONTENT OF F_4 WHITE SEEDED SEGREGATES FROM A CROSS OF GARNET × I-28-46, GROWN AT FALLIS, IN 1931

Category	Mean protein content
Vitreous	13.0 ± 0.25
Semi-vitreous	11.0 ± 0.27
Semi-starchy	9.4 ± 0.44
Starchy	9.2 ± 0.16

These data show that there is a progressive decrease in the mean protein content of the samples, with an increase in starchiness. The test of independence between kernel texture and protein content for the above material gave a P value of less than 0.01. This clearly demonstrates the high degree of association between these two characters for the material when grown under Fallis conditions.

Discussion

The importance of some of the problems connected with securing an indication of the quality of hybrid material in early generations has been discussed by Aamodt and Torrie (2). They have suggested the growing of the material on the gray soils in an environment such as that at Fallis, Alberta, where a good differentiation in kernel texture is obtained. They show by correlation studies that this character is of considerable value as a means of discarding hybrid lines of low quality. In conjunction with the above studies, the present experiments were carried out to determine the heritable nature of, and the relation between, protein content under different environments for several series of crosses.

The heritable nature of kernel texture, shown in the crosses studied, is in agreement with the conclusions of other workers studying hybrid material (3–5, 7, 8, 10, 13, 15–17). The important effect of environment on the expression of kernel texture, as shown by the results obtained at Edmonton and Brooks, is in agreement with the statements of Roberts and Freeman (23) and Hayes (17). This effect clearly indicates the necessity of growing the material under differential environment, such as at Fallis, that will permit the expression of the genetic factors governing kernel texture.

Protein content is also a character of considerable value in preliminary determinations of baking quality. The polymeric factor hypothesis for the

mode of inheritance of this character is in agreement with the results obtained by Clark and Hooker (11). The great influence that environmental factors, such as climate and soil type, have on protein content agrees with the results reported by Clark and his co-workers (10, 12). The results show that the genetic factors governing this character can best be determined in an environment which gives the maximum phenotypic expression for each genotype.

The relation between kernel texture and protein content is highly significant for all the crosses studied. In the case of the crosses of Reward with Selections I-28-46, I-28-60 and I-28-62, where differences in kernel texture were masked at Edmonton by the environment, the differences in protein content of the starchy and vitreous groups were significant, in both the F_2 and F_3 in all cases save one.

The heritable nature of the two characters of kernel texture and protein content, and their relations with loaf volume and partial baking score, indicate that they are of considerable value as indices of baking quality. The simplicity of the kernel texture method, as shown by Aamodt and Torrie (2), when tested on material grown at Fallis, and its much closer relation with baking quality determined on material grown at Edmonton, than that of protein content, indicates that kernel texture is the better index for baking quality. The importance of growing the material to be tested under an environment which will give a differential reaction for kernel texture every year cannot be overemphasized.

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STUDIES ON BROWNING ROOT ROT OF CEREALS[1]

III. PHOSPHORUS-NITROGEN RELATIONS OF INFESTED FIELDS
IV. EFFECTS OF FERTILIZER AMENDMENTS
V. PRELIMINARY PLANT ANALYSES

By T. C. Vanterpool[2]

Abstract

These papers deal with the elucidation of the factors which predispose wheat seedlings following summerfallow to *Pythium* root rot in certain parts of Saskatchewan. The chemical soil analyses on 66 sample pairs reveal that available phosphorus in the soil is lower and nitrate nitrogen higher in browning patches than in normal patches. Greenhouse experiments and field observations show that phosphate amendments at the time of seeding, and farmyard manuring, permit the development of normal plants in browning soil; cereal straw applications inhibit the disease to some extent, while inorganic nitrogenous fertilizers usually have no effect or occasionally may prove detrimental. Preliminary plant analyses indicate that the total phosphorus in diseased plants is lower than in healthy ones, and that there is relatively more water-soluble nitrogen and relatively less protein nitrogen in diseased than in healthy plants.

The soil and crop-culture factors, especially the practice of bare summerfallowing, which may influence the available phosphorus-nitrate nitrogen ratio in the soil, are discussed in relation to disease incidence. It appears that any crop practice which tends to increase the available phosphorus-nitrate ratio in the soil inhibits the disease, and conversely. This is used as a basis for control recommendations.

Attention is drawn to the striking similarity of the factors affecting the severity of *Pythium* root rot of wheat and *Pythium* root rot of sugar cane in Hawaii.

It is considered that an improper balance of available phosphorus and nitrate nitrogen in the soil leads to an unbalanced metabolism of the wheat seedlings at a critical stage in their development, thereby predisposing them to fungal attack. The effect of the unbalanced nutrients on the parasitic vigor of the pathogen still requires elucidation.

III. PHOSPHORUS-NITROGEN RELATIONS OF INFESTED FIELDS

The parasitic nature of browning root rot was demonstrated in a previous communication (46) and its severity on the wheat crop following summerfallow was pointed out. It is caused primarily by species of *Pythium* (of which *P. arrhenomanes* Drechs. is probably the chief) which attack the root tips and fine laterals during the first few weeks of growth and by the fifth or sixth week produce a yellowish-brown discoloration of the outer leaves and a retardation of growth. The shift in location from one year to another of severely diseased zones in central and north-central Saskatchewan demonstrates clearly the close correlation of the disease with environmental factors and that these are of more importance than crop sequence in determining its severity. Thus since the browning root-rot epidemic of 1928, the yearly magnitude of losses has varied but little over the province as a whole. These papers present a general consideration of the problem together with data on the relation of soil fertility and plant nutrition to disease incidence.

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General Considerations

Evidence from our own findings and observations, and from the work of others, made it reasonable to postulate that unbalanced soil nutrients possibly increased the liability of the seedlings to attack from the soil-borne parasites at a critical stage in their development. A general consideration of the factors likely to be involved, as they appertain to prairie conditions, will place the problem in its true perspective.

Nitrogen is probably the soil nutrient most influenced by summerfallowing. The work of Wyatt and co-workers (49) and of Newton (30) showed that nitrate nitrogen in prairie soils is highest during May and early June on land which was summerfallowed the previous season. Richardson and Fricke (37) reported that in Australia there is a negative response of wheat after bare fallow to nitrogen applications owing to the accumulation of nitrate sufficient for or in excess of the heavy demands of the fallow crop. Ellis (14) also found that on the open prairie soils of Manitoba no positive response of wheat on fallow was obtained from sodium nitrate or ammonium sulphate. The thorough cultivation increases the activities of the nitrifying micro-organisms and thus ensures a greater accumulation of nitrate. Bedford (4) considered that the potentiality of semi-arid prairie soils for rapid nitrification can best be explained by estimating the bacterial numbers not on the soil aggregate, but on the organic matter as a unit. Land bearing a second crop of wheat is relatively low in nitrate (30, 37, 49), which has been removed by the previous crop and by micro-organisms in the decomposition of the crop residue. Richardson and Fricke (37) also found that in Australia, wheat on stubble responded to nitrogenous fertilizers. Recent work on the fertilization of the Canadian prairies is in general agreement with the findings of the Australian workers. In this connection it should be noted that *Pythium* root rot is most severe in June on wheat on well worked fallow, and is seldom of any importance on the stubble crop (45, 46). In Hawaii, Carpenter (6, 7) put forward the contention that an excess of nitrogenous fertilizers rendered the sugar cane more susceptible to root rot by the same group of fungi which causes browning root rot of cereals. Also, that the damage was considerably lessened when sugar cane fibre, almost entirely cellulose, was previously incorporated with the soil. That is, susceptibility of cane roots to attack of *Pythium* might be acquired or lost depending on the quality or balance of nutrients. This doubtless involves the complex questions of the carbohydrate-nitrogen ratio and the biological activities of soil organisms.

There are numerous references in plant disease literature, many of them, it must be confessed, without detailed supporting data, which indicate generally that excess nitrogenous fertilizers render plants more susceptible to disease, providing phosphate or potash or both are present in limited amounts. A general reference on these points need only be made to the literature compilations of Wilson (48, pp. 164-165). It is not likely that there is a deficiency of potash in the open prairie soils of Saskatchewan (22), but in many districts there is a limited amount of available phosphorus as shown by chemical

determinations, and the marked response of wheat on fallow to phosphate fertilization (29). Results of a similar nature with regard to phosphorus have been obtained in the adjoining province of Alberta by Wyatt and Ward (50), and in Manitoba by Ellis (14). Nygard (31) reported a positive response of spring wheat to phosphates on many soils in Montana. The suggestion arose that a low content of available phosphate may in some way be contributory to the *Pythium* root rot. It is an interesting fact that the amount of available phosphorus in a given prairie soil appears to be fairly constant throughout the year, as indicated by Mitchell (29). Our findings on a small number of fields are in agreement with this (Table VIII). However, Emmert (15), Meyer (28) and Sturgis (42) have indicated that a correlation might exist between available phosphorus and organic matter in the soil, the state of decomposition of the latter being the chief determining factor. Richardson and Fricke (37) have pointed out that it is the customary practice in wheat-growing sections in Australia to apply phosphate to wheat following fallow. The increasing use of phosphate fertilizers on the Canadian prairies during the last few years indicates that the same will soon be true for wheat culture in the Canadian West.

The tendency for phosphorus to decrease soil nitrification, as indicated by several workers, might have some bearing on the problem. According to Wyatt and Ward (50) the application of phosphates to certain soils in Alberta tended to decrease nitrification. Ivanovskaia (21) found that in alkaline soil highly resistant to oxidation, water-soluble phosphorus pentoxide decreased when the nitrate content was high and increased when it was low.

There is also the possibility that there exists an antagonistic or a reciprocal relation between phosphate and nitrate availability or accumulation in the soil and their absorption by the plant (19, 20, 24, 25). Hoagland (19) has pointed out that, "Under appropriate conditions of solution technique, in experiments with plants such as wheat or barley, it may be shown that there exists a reciprocal relation between nitrate and phosphate. By decreasing the concentration of phosphate in the culture medium at certain stages of growth, the plant may be caused to accumulate more nitrogen. Conversely, by decreasing the concentration of nitrate, plants may sometimes be caused to accumulate a very high content of phosphate". In general, the findings of MacGillivray (24), McCool and Cook (25), Huelsen (20), and Chapman (9) support the same views. McCool and Weldon (26), Davidson (12), Beaumont (3) and Chapman (9), obtained indications that nitrate influenced the availability of phosphorus in the soil. Breazeale (5), however, could find no reciprocal relation between nitrogen and phosphorus in solution cultures. Smith and co-workers (41) reported that sodium nitrate applications increased the amount of available phosphorus in the soil from 12.5 p.p.m. to 18.6 p.p.m. in a Carrington loam in Iowa. The investigational results of Pearsall and Ewing (33), Cook (10), Emmert (15), Pettinger (34), Sessions and Shive (39), and Chapman (9), are in general agreement with this view. Thomas (43), citing from the work of Sekera (38), in summarizing the current

view stated that "All experimental evidence appears to indicate that the plant, especially in the early stages of growth up to blooming, absorbs the nutrient ions in approximately the same proportion that these ions are present in the nutritive medium". It would appear then generally that, under conditions of unbalanced phosphate and nitrate in the nutrient medium, the anions tend to have an antagonistic or a reciprocal effect on the rate of absorption of each other. Under more balanced conditions, especially during the first few weeks of growth, the phosphate and nitrate are found in the cell sap in relatively the same proportions as in the external solution.

The work of Cooke (11) on the relation of high phosphate-fixing capacity of Hawaiian soils to *Pythium* root rot of sugar cane, and that of Heck (17) on the relation of low phosphorus and high mineral nitrogen to the same disease, have a direct bearing on the browning problem, but will not be discussed at this point as they were published after these investigations were completed. The fact that Heck's interesting results and those submitted in this paper were arrived at independently should add weight to the conclusions presented in both papers. The similarity of the nutrient factors affecting *Pythium* root rot of wheat and of those affecting *Pythium* root rot of sugar cane as reported by Cooke (11), Heck (17), and more recently by Carpenter (8), are pointed out in the general discussion.

In view of the above considerations regarding unbalanced nitrogen and phosphorus metabolism, it seemed advisable to analyze soil samples from browning-infested patches and apparently healthy patches in the same field for nitrate nitrogen and available phosphorus. In conjunction with this a study of the effects of nitrogenous and phosphatic fertilizers mainly, on wheat seedlings grown in crocks in browning or "diseased" and in normal or "healthy" soils, was also conducted. In this paper "diseased" soil and "healthy" soil shall be taken to mean soil which produces diseased and relatively healthy seedlings respectively.

Available Phosphorus and Nitrate Nitrogen of *Pythium*-infested Fields

The collection of soil samples from areas with diseased plants and from areas of healthy plants began with the first appearance of the disease in June. Samples were obtained only from fields showing characteristic pronounced symptoms of the disease. These were situated over a wide range of territory in central and north-central Saskatchewan. A thorough washing and examination of the roots in a large white porcelain dish facilitated and insured a correct diagnosis in the field. Each soil sample for analysis was a composite of eight samples taken to a depth of six inches from between the rows and thoroughly mixed on an oilcloth. Thus, from each study field one composite sample of soil from diseased and one from apparently healthy areas were obtained. The samples were placed in quart sealer jars and 4 cc. of toluene added to each, in order to stop nitrification and other bacterial action. The samples were thoroughly air dried in the laboratory within 48 hours of

collecting, and then stored in the jars until the chemical analyses were performed about a fortnight later. It was considered that no appreciable change in the nitrate nitrogen content would occur in the air-dried soil. A slight modification of the Truog method, as employed by Mitchell (29), was used for the estimation of available phosphorus, and Harper's modification (16) of the Colorimetric Phenoldisulphonic Acid method for the estimation of nitrate nitrogen.*

Table I shows the results of analyses of soil samples from diseased and healthy areas of wheat on summerfallowed land for June, 1932, and Table II the results for June, 1933.

TABLE I

ANALYSES OF SOIL SAMPLES FROM DISEASED AND HEALTHY AREAS OF WHEAT ON SUMMERFALLOWED LAND, JUNE 1932

Field	Locality (town)	Date collected	Available P, p.p.m.		NO_3, p.p.m.		pH, Electrometric		Soil texture
			Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	
1	Saskatoon	June 14	36	78	17.2	10.9	7.30	7.92	Loam, silty
2	Environ	14	55.5	82	12.9	1.7	7.07	8.02	Loam
3	Wilkie	17	23.5	52	17.8	7.5	7.36	8.17	Loam
4	Scott	17	15	42	16.6	1.6	7.33	7.85	Loam
5	Saskatoon	18	19	56	4.4	11.6	6.67	8.07	Loam
6	Bruno	20	53	103	1.2	1.1	7.42	7.93	Heavy loam
7	Kinistino	21	82	139	4.6	3.9	6.95	7.26	Heavy loam
8	Aberdeen	21	15	63	14.1	6.8	7.65	8.01	Loam
9	Prudhomme	21	38	49	10.8	1.2	7.71	7.42	Loam, silty
10	Dana	21	31.5	77	24.2	23.4	7.20	7.62	Loam, silty
11	Bremen	21	34.5	60	25.7	7.8	—	—	Loam, silty
12	Wakaw	21	20.5	64	4.1	1.4	—	—	Loam, silty
13	Domremy	21	42	72	5.1	2.5	—	—	Loam, smooth
14	Duck Lake	22	23	54	4.3	0.7	—	—	Loam, smooth
15	Waldheim	22	25	49	13.2	2.0	—	—	Loam, light
16	Sutherland	27	90	84	2.3	4.4	—	—	Clay loam
17	Saskatoon	27	32	80	3.4	2.2	—	—	Clay loam
18	Saskatoon	27	30.5	45.5	2.4	2.6	—	—	Loam
19	Lanigan	28	53	51.5	0.3	0.2	—	—	Loam, heavy
20	Lanigan	28	35	52	0.1	0.2	—	—	Fine sandy loam
21	Lanigan	28	24	28	0.3	0.3	—	—	Fine sandy loam
22	Humboldt	28	24	49	4.6	0.6	—	—	Loam
Mean			36.5	65.0	8.6	4.3	7.26	7.82	

The results in Tables I and II are, in the main, similar and may be considered together. In both it will be observed that, with few exceptions, available phosphorus is less in diseased areas than in healthy, while nitrate nitrogen is greater in diseased areas than in healthy. This relation may not have prevailed at the time of seeding, but relevant work (9, 10, 38, 39, 43) supports the view that plants absorb nutrients during the early stages of growth in approximately the same proportion in which these elements are present in

These determinations were made by Frank Riecken, M.Sc., formerly of the Soils Department, to whom my thanks are due.

TABLE II

ANALYSES OF SOIL SAMPLES FROM DISEASED AND HEALTHY AREAS OF WHEAT ON SUMMEPFALLOWED LAND, JUNE, 1933

Field	Locality (town)	Date collected	P, p.p.m.		NO_3, p.p.m.		pH		Soil texture
			Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	
1	Saskatoon	June 6	29	40	6.0	5.9	7.70	7.60	Loam
2	Saskatoon		30	59	10.9	4.3	6.80	6.75	Loam
3	Saskatoon	June 8	56	160	24.0	24.4	8.10	7.64	Clay
4	Saskatoon		36	39	8.7	7.3	7.42	6.80	Loam
5	Saskatoon		28	46	12.0	16.0	7.15	7.44	Loam
6	Saskatoon		23	17	17.2	9.6	6.00	7.60	Loam
7	Saskatoon	June 9	64	33	30.0	27.7	6.44	7.62	Loam (heavy)
8	Saskatoon		54	78	11.4	10.1	6.30	7.80	Loam
9	Vanscoy		38	47	36.2	38.0	6.65	7.07	Loam
10	Saskatoon		28	57	25.0	19.7	6.30	7.70	Loam (silty)
11	Clavet	June 13	33	63	20.2	9.2	6.35	7.70	Clay loam
12	Elstow		47	96	19.7	18.5	6.36	7.16	Silty clay
13	Lanigan		34	37	3.0	1.3	7.90	7.90	Loam
14	Sheho		37	56	4.5	3.4	8.00	7.74	Loam
15	Yorkton		31	81	17.5	5.6	8.08	8.10	Hy. silt loam
16	Yorkton		41	43	30.4	19.9	8.00	7.10	Hy. silt loam
17	Yorkton		24	62	7.1	1.8	8.00	8.16	Loam
18	Yorkton		18	36	9.3	2.6	8.30	8.10	Loam
19	Quill Lake	June 14	27	41	4.3	4.5	7.45	7.76	Loam
20	?		32	64	10.6	6.0	7.76	7.77	Loam
21	Englefeld		24	46	3.1	3.0	8.08	7.50	Light loam
22	St. Gregor		33	35	15.9	13.4	7.92	7.63	Loam
23	Munster		31	41	27.3	33.9	7.77	7.75	Loam
24	Humboldt		27	39	19.8	5.7	6.91	6.85	Loam
25	Humboldt		31	68	29.8	12.9	6.33	7.52	Loam
26	Aberdeen	June 15	28	56	5.9	5.9	8.17	8.10	Clay loam
27	Saskatoon	June 17	33	68	15.4	10.2	6.92	7.45	Loam
28	Saskatoon		23	42	22.4	32.7	5.90	6.40	Loam
29	Sonningdale		26	38	24.8	18.9	6.15	6.59	Loam
30	Sonningdale		26	62	29.5	13.8	6.14	7.85	Loam
31	Sonningdale		23	23	15.3	3.5	6.34	6.59	Loam
32	Sonningdale		25	53	25.8	22.5	6.51	6.75	Loam
33	Scott	June 20	15	14	21.6	5.0	5.50	5.75	Heavy loam
34	Unity		14	44	15.2	9.8	5.55	7.10	Loam
35	Unity		16	18	28.8	9.2	5.40	5.45	Loam
36	Unity		17	40	22.5	62.0	5.49	6.25	Loam
37	Wilkie		18	51	33.0	43.5	5.65	7.35	Loam
38	Brandon		65	48	44.0	13.4	7.85	8.10	Clay loam
39	Prudhomme	June 22	34	41	22.2	16.4	7.93	7.85	Loam
40	Dana		20	50	92.5	15.9	6.50	7.61	Loam
41	Wakaw		26	49	6.2	22.2	6.95	7.85	Loam (silty)
42	Domremy		35	68	12.9	2.6	6.93	7.60	Loam (silty)
43	St. Isidore		34	48	3.8	7.5	7.35	7.50	Loam
44	Rosthern		18	64	13.1	13.2	6.20	7.62	Heavy loam
Mean			30.7	51.4	19.5	14.4	6.95	7.37	
Mean, 1932-1933			33.6	58.2	14.1	9.4	7.10	7.59	

the soil. It is, however, reasonable to expect a greater number of irregular results with nitrates than with available phosphorus, as shown in Tables I and II, because of the greater tendency of nitrates to fluctuate with changing biological and physical conditions of the soil and with the movement of the

soil moisture. The pH of the soil solution from diseased areas is usually slightly lower than that from healthy areas, but no importance is attached to this difference. All of the results appear to be independent of soil type. The available phosphorus and nitrate nitrogen results are presented graphically in Figs. 1 and 2.

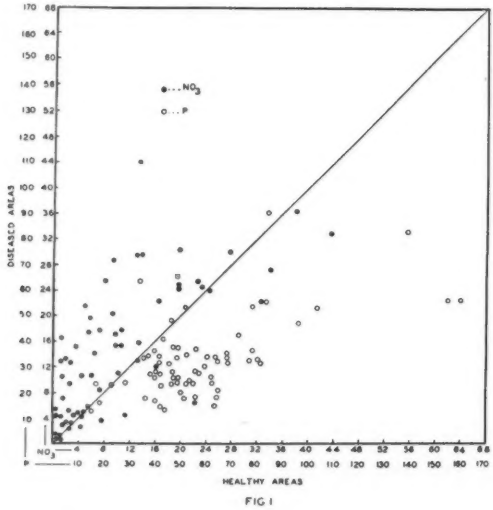

FIG. 1

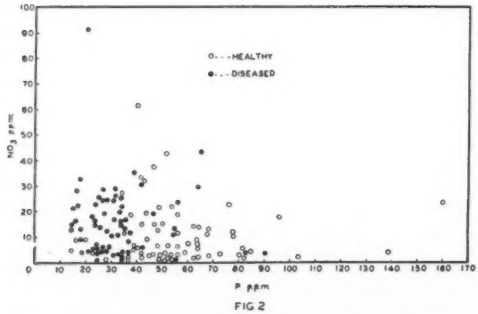

FIG. 2

FIGS. 1 and 2. *Showing graphically the distribution of the fields according to the results in Tables I and II.*

The presence of soluble salts in the soil solution is considered by McGeorge and Breazeale (27) to depress phosphate solubility, and Sideris and co-workers (40) have mentioned that in high salt concentrations pineapples use the oldest leaves as storage organs for the excess of salt. However, tests showed soluble salts to be present in very small amounts in the soil samples of Table II.

Fields in Relation to Available Phosphorus and Nitrate Nitrogen Ratios

In Table III, the 66 fields are arranged according to their relative content of available phosphorus and nitrate nitrogen as shown in Tables I and II. In 65% of the fields the diseased areas contain less available phosphorus and

TABLE III

NUMBER OF FIELDS ACCORDING TO AVAILABLE PHOSPHORUS AND NITRATE NITROGEN RATIOS OF THE SOIL IN DISEASED AND HEALTHY WHEAT PATCHES FOR 1932 AND 1933

	Ratio	No. of fields in which available phosphorus in			
		D* < H	D > H	D = H	
No. of fields in which nitrate nitrogen in	D > H	43	5	1	49
	D < H	14	1	—	15
	D = H	2	—	—	2
		59	6	1	66

*D *represents diseased wheat areas, and* H, *healthy wheat areas.*

more nitrate nitrogen than healthy areas; 21% show low available phosphorus associated with low nitrate nitrogen; 7.5% show high available phosphorus associated with high nitrate nitrogen, and only 1.5% show high available phosphorus associated with low nitrate nitrogen. Ninety per cent of diseased fields are lower in available phosphorus than healthy fields, and 74% of diseased fields are higher in nitrate nitrogen than healthy fields. Thus viewed, the results indicate more clearly the probable correlation between less available phosphorus and more nitrate nitrogen in browning than in normal areas, and suggest that low available phosphorus is possibly more closely correlated with the disease than high nitrate, if the two can be considered separately. As already pointed out, however, the nitrate differences might have been greater during the first four weeks of growth before disease symptoms appeared.

Nutrients in Browning Fields as a Percentage of Nutrients in Healthy Fields

When the nutrients in each browning field are expressed as percentages of the nutrients in each normal field and the mean taken, it is found that for 1932, the amount of available phosphorus is 56.5% and of nitrate nitrogen 324.3%; and for 1933, 67.1% and 188.1% respectively. These are shown diagrammatically in Fig. 3. They suggest a possible reciprocal relation between available phosphorus and nitrate nitrogen; an increase in available phosphorus being accompanied by a decrease in nitrate nitrogen and *vice versa*.

Such a relation has been recognized by many investigators (19, 20, 24, 25). It should be borne in mind, however, that nitrates might fluctuate a great deal and frequently accumulate to a great extent in soils that are high in available phosphorus.

Soil Nutrients and Plant Growth

In Table IV are shown the relative heights of seedlings in June, in diseased and healthy areas, together with the corresponding available phosphorus and nitrate nitrogen of the soil. The average height of the diseased seedlings is 7.8 cm. less than that of healthy seedlings (those sections receiving phosphate applications in Fields 34 and 35 are not included), the diseased condition being associated, as before, with less available phosphorus and more nitrate nitrogen than the healthy condition. In Fields 5 and 33, when the diseased areas of the fallow crop are compared with the healthy areas of the stubble

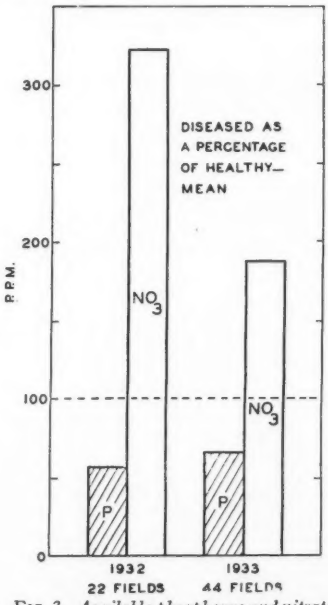

FIG. 3. *Available phosphorus and nitrate nitrogen in browning fields expressed as percentages of available phosphorus and nitrate nitrogen in normal fields, 1932 and 1933.*

TABLE IV

HEIGHT OF WHEAT SEEDLINGS AT APPROXIMATELY SIX WEEKS IN RELATION TO AVAILABLE PHOSPHORUS AND NITRATE NITROGEN CONTENT OF SOIL AND TO CULTIVATION PRACTICE, JUNE 1933

Field	Location (town)	Treatment	Condition	Height, cm.	P, p.p.m.	NO_3, p.p.m.
2	Saskatoon	Fallowed	Healthy	33	59	4.3
		Fallowed	Diseased	23	30	10.9
5	Saskatoon	Fallowed	Healthy	34	46	16.0
		Fallowed	Diseased	24	28	12.0
		Stubble crop	Healthy	31	30	3.5
31	Sonningdale	Fallowed	Healthy	24	23	3.5
		Fallowed	Diseased	20	23	15.3
33	Scott	Fallowed	Diseased	22	15	21.6
		Spring ploughed	Healthy	22	14	5.0
34	Unity	Fallowed	Healthy	30	44	9.8
		Fallowed	Diseased	26	14	15.2
		Ploughed in autumn before fallow	Healthy	33	31	4.1
		Fallow + P	Healthy	42	—	—
35	Unity	Fallowed	Diseased	22	16	28.8
		Fallowed + P	Healthy	38	18	9.2
41	Wakaw	Fallowed	Healthy	37	49	22.2
		Fallowed	Diseased	29	26	6.0
42	Domremy	Fallowed	Healthy	42	68	2.6
		Fallowed	Diseased	30	35	12.9
43	St. Isidore	Fallowed	Healthy	37	48	7.5
		Fallowed	Diseased	27	34	3.0
44	Rosthern	Fallowed	Healthy	31	64	13.2
		Fallowed	Diseased	22	18	13.1

crop, the available phosphorus content of the soil in the respective areas is seen to be practically the same, while the nitrate nitrogen is high in both cases in the diseased fallow-crop area, *i.e.*, the closer P/N ratio in both cases is associated with the diseased condition.

Special attention should be drawn to Fields 31 and 34. In Field 31, the diseased area was bare fallowed during the whole summer of 1932, while the healthy area carried a crop of wheat and oats in 1932 which was cut for green feed in mid July. The latter area was not ploughed until mid August. The difference in the condition of the seedlings on the two portions of the

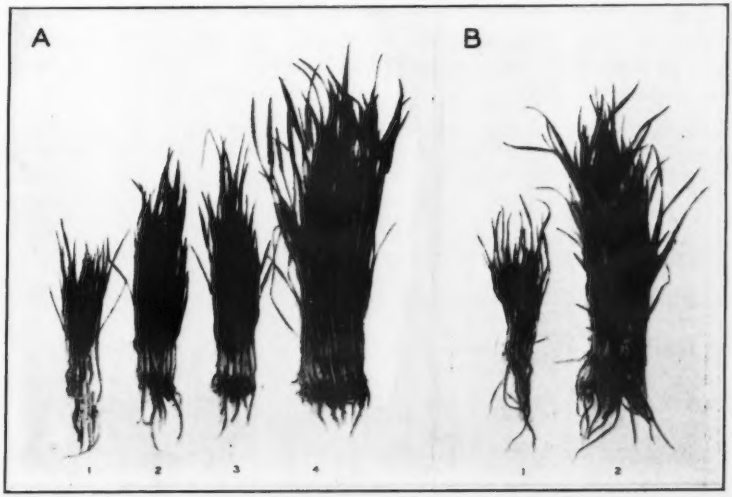

FIG. 4. *Wheat following summerfallow; 20 plants to each bundle.* A, *field 34, Table IV, 1, plants showing browning symptoms; 2, normally healthy plants; 3, healthy plants from strip on which weeds were ploughed under the autumn preceding summerfallow, and 4, triple superphosphate treated plants.* B, *field 35, Table IV, 1, untreated diseased plants, and 2, plants receiving triple superphosphate.*

same field could clearly be observed from some distance. In Field 34, all portions received the same fallow treatment in 1932, but one wide strip was ploughed shallow in the autumn of 1931 to turn under a heavy growth of weeds. This strip carried a uniformly healthy crop in 1933, while the remainder of the field, with the exception of the strip receiving phosphate fertilizer, was spotted with diseased and healthy areas (Fig. 4). It is possible that decomposing organic matter of the "weedy" strip with its concomitant evolution of carbon dioxide increased the availability of phosphorus as suggested previously (15, 28, 42), while the nitrogen removed by the weeds produced a relative decrease in the available nitrate in the soil (2).

Relation between Soil Nutrients, Height at Maturity, and Date of Maturity

Eleven of the fields selected for study in June, 1932, *viz.*, numbers 1, 2, 5, 6, 8 to 10, 15 to 18 (Table I), were visited in August just before harvest and, with the exception of Field 16, the diseased wheat was a few to 20 cm. shorter and from 3 to 21 days later in maturing than the healthy wheat. Similar conditions obtained in fields under observation in 1933 and 1934. This delayed maturity might be attributed, in part at least, to the higher nitrate content of the soil. Field 15 (Table V) showed a slight difference in height but there was no appreciable difference in date of maturity. In the case of Field 16 (Table I), the exception referred to above, there was no difference in height of plants or in date of maturity between what were diagnosed in June as diseased and as healthy patches, respectively. Data on four of these fields are presented in Table V.

TABLE V

RELATION BETWEEN AVAILABLE PHOSPHORUS AND NITRATE NITROGEN CONTENT OF SOIL, HEIGHT OF PLANTS AT MATURITY, AND DATE OF MATURITY, 1932

Field	Locality (town)	Crop condition in June	Soil analyses, June		Height on Aug. 12, cm.	Estimated delay in maturity of diseased plants
			P, p.p.m.	NO_3, p.p.m.		
8	Aberdeen	Healthy	63	6.8	82	3 to 4 days
		Diseased	15	14.1	77	
9	Prudhomme	Healthy	49	1.2	101	10 to 14 days
		Diseased	38	10.8	84	
10	Dana	Healthy	77	23.4	99	14 days
		Diseased	31.5	24.2	79	
15	Waldheim	Healthy	49	2.0	99	No appreciable difference
		Diseased	25	13.2	96	

In general, it appears that a narrow P/N ratio in the available soil solution early in the season allows the disease to become manifest and delays maturity, whereas a wide P/N ratio maintains the crop in apparently healthy condition and hastens maturity. At the same time it might be possible to have a narrow ratio in a normal soil which was high both in available phosphorus and nitrate without the seedlings becoming susceptible to fungal attack. Since both diseased and healthy areas in each field have received the same cultural treatment, it may be inferred that the differences in available nutrients are due to inherent soil factors or to the activities of micro-organisms.

Expected Response to Phosphatic Fertilizers

The question arises, can an increase in yield from phosphate applications be expected on the browning areas which are relatively low in available phosphorus? Mitchell (29) concluded from his findings for Saskatchewan soils generally "that the soils lighter than clays where the "availability" of

phosphorus is below 20 parts per million as found by this method (modification of Truog's) large increases in grain yield may be expected from phosphate treatments of the soil. With an availability of from 20 to 40 parts per million a response is still probable, while at 50 to 60 parts per million "available" phosphorus, the point is reached where little or no response is likely". On the basis of his findings, the data in Tables I and II have been arranged to show the distribution of fields in relation to expected yields from phosphate applications based on available phosphorus in the soils. Three arbitrary divisions have been recognized in accordance with Mitchell's data. They are considered significant only in so far as they allow us to say that a soil is well supplied or moderately supplied with available phosphorus or is deficient in it. All fields with more than 45 p.p.m. available phosphorus are considered *unlikely* to give increases in yields; from those with 21 to 45 p.p.m. available phosphorus, increases in yields are *probable*, and from those with less then 20 p.p.m. available phosphorus, *large* increases in yields may be expected.

Table VI gives the distribution of fields in relation to expected yields from phosphate applications based on available phosphorus in soils, and Table VII presents the percentage distribution.

TABLE VI

DISTRIBUTION OF FIELDS IN RELATION TO EXPECTED YIELDS FROM PHOSPHATE APPLICATIONS BASED ON AVAILABLE PHOSPHORUS IN SOILS

Year	Healthy areas			Diseased areas			Total no. of fields
	Increase in yields			Increase in yields			
	Large. (P, p.p.m. 20 or less)	Probable. (P, p.p.m. 21 to 45)	Unlikely. (P, p.p.m. 46 or more)	Large. (P, p.p.m. 20 or less)	Probable. (P, p.p.m. 21 to 45)	Unlikely. (P, p.p.m. 46 or more)	
1932	0	2	20	3	14	5	22
1933	3	16	25	8	31	5	44
1932-1933	3	18	45	11	45	10	66

TABLE VII

PERCENTAGE DISTRIBUTION OF FIELDS IN RELATION TO EXPECTED YIELDS FROM PHOSPHATE APPLICATIONS BASED ON AVAILABLE PHOSPHORUS IN SOILS

Year	Response to P likely. P, 45 p.p.m. or less		Response to P problematical. P, 46 p.p.m. or more	
	Healthy	Diseased	Healthy	Diseased
1932	9.1	77.3	90.9	22.7
1933	43.2	88.6	56.8	11.3
1932–1933	31.8	84.8	68.1	15.1

It will be seen from the 66 fields studied that the greatest response to phosphate applications is likely to occur in the diseased areas, *viz.*, 84.8%, while only 31.8% of the healthy areas are likely to give increases in yield. These results aid in explaining: first, the general uniformity in wheat fields in browning root rot districts which have received phosphate fertilizer, compared with the unevenness in height and stage of maturity in those untreated; and second, the results of the fertilizer experiments conducted on browning and normal soil in the greenhouse and reported in Section IV.

It is recognized that the method of ascertaining the "available" phosphorus in a given soil and using this as a guide in predicting the probable response to phosphate of a wheat crop grown thereon has decided limitations and is not infallible, but the results in this province and elsewhere have more often than not shown a close correlation. In recent years there have been rapid developments of both chemical and biological methods for diagnosing nutrient deficiencies in soils and of basing recommendations for the use of fertilizers on the results obtained. The Truog method of ascertaining the "available" phosphorus in a given soil has provided, in a large majority of cases, a fair basis for predicting the probable response to phosphate fertilizers.

Browning Root Rot in Relation to Cultivation Practices and Available Phosphorus and Nitrate Content of the Soil

The fields chosen for this study were partly in fallow crop and partly in a fall- or spring-ploughed stubble crop. The burned straw-stack area and the gray soil area were in zones of fallow crop. The results of the determinations are given in Table VIII.

TABLE VIII

ANALYSES OF SOIL SAMPLES IN RELATION TO VARIOUS CULTIVATION PRACTICES AND *Pythium* ROOT ROT OF WHEAT

Locality (town)	Date collected	Soil preparation	Condition of crop	"Available" P, p.p.m.	NO_3, p.p.m.
1932:					
Sonningdale	June 14	Following fallow	Diseased	38	10.6
		Following wheat—fall ploughing	Healthy	35	0.9
		Burned straw-stack zone—fallow, 1931	Healthy	129	2.3
Scott	June 17	Following fallow	Diseased	15	16.6
		Following wheat—spring ploughing	Healthy	19	0.6
		Spot of gray soil—fallow, 1931	Healthy	42	1.6
1933:					
Saskatoon	June 8	Following fallow	Diseased	28	12.0
		Following wheat	Healthy	30	3.5
Scott	June 20	Following fallow	Diseased	15	21.6
		Following wheat—spring ploughing	Healthy	14	5.0

The results show that in the same field the amount of available phosphorus is practically the same in the "diseased" fallow-crop areas as in the normal fall-or spring-ploughed areas (*cf.* 29), while the nitrate nitrogen is considerably higher in the "diseased" fallow-crop areas. That is, where the P/N ratio is relatively wide the crop is apparently healthy, and conversely where it is narrow the crop is diseased. Thus it appears that cultivation practices can have a determining influence on the incidence of the disease.

In the Sonningdale field where the burned straw and chaff ashes have returned much available phosphorus to the soil, a healthy crop develops. The wide P/N ratio prevailing in the gray spot in the Scott field also permits a healthy condition.

It is possible that the nitrate in the diseased areas was not initially higher than in the healthy, but became so because of the incapability of the damaged root system to absorb and utilize efficiently the nitrate already present. However, this does not seem likely in these instances as fallowed soil is invariably higher in nitrate than stubble soil. Similarly, it could be argued that the healthy plants would also tend to absorb more phosphorus and reduce the available supply relative to the diseased area, but there is still ordinarily about twice as much available phosphorus in the healthy area in spite of the greater absorption (Table I).

Burned Straw-stack Areas

The better growth and yield on areas which formerly carried straw stacks which were subsequently burned is carried over for many years in most cases. Soil samples from three such zones were included in the analyses, the results of which are presented in Table IX.

TABLE IX

ANALYSES OF SOIL SAMPLES FROM OLD BURNED STRAW-PILE ZONES. WHEAT FOLLOWING FALLOW

Locality (town)	Condition of crop	"Available" P, p.p.m.	NO_3, p.p.m.	pH
1932:				
Sonningdale	Healthy	129	2.3	8.11
1933:				
Saskatoon	Healthy	220	9.2	8.60
Saskatoon	Healthy	126	12.4	7.50
Mean		158	7.9	8.07

The results indicate that the good growth is to be accounted for by the extremely high available phosphorus content of the soil, coupled with a sufficiency of the other essential elements, and not by a high nitrate content as is commonly assumed on the prairies. The nitrate is usually of an order comparable to that of many normally healthy areas in the same field. The

kind of cereal doubtless has an influence on the amount of phosphorus present; for example, wheat straw and chaff will supply more phosphorus than oat straw and chaff (18). As reported later under the section on fertilizer treatments, wheat in browning soil, which is naturally lower in available phosphorus and higher in nitrate nitrogen than normal soil, responds well to cereal-straw ashes, while in the normal soil there is little or no response.

Miscellaneous Considerations

1. *Dark- and light-colored soil areas.* In some browning root-rot districts, especially in north-central Saskatchewan, there is considerable variation in soil color in the same field due mainly to the removal of some surface soil by wind erosion or to the existence at one time of lightly wooded areas. To ascertain whether there was any correlation between these areas and browning root rot, 35 fallow fields, or parts thereof, were charted for dark- and light-colored areas in the autumn of 1932. An examination of the young wheat crop the following June showed no definite correlation between color and the presence of the disease except in two instances. In general, therefore, there seems to be no relation between soil color and the disease in a given field.

2. *Iron and manganese.* It is well known that many alkaline soils have a tendency to fix iron and manganese so that these elements become almost unavailable to plants. In preliminary pot experiments on the application of iron and manganese compounds to browning root-rot soil no difference in the growth of wheat could be recognized between the treated plants and the checks.

IV. EFFECTS OF FERTILIZER AMENDMENTS

The ratios of available phosphorus and nitrate nitrogen in browning and normal soil, as revealed by the chemical analyses, made necessary a study of the effects of fertilizers, especially phosphatic and nitrogenous, on browning root rot of wheat. Consequently, throughout this section on fertilizers it should be borne in mind that browning or "diseased" soil is relatively lower in available phosphorus and higher in nitrate nitrogen than normal or "healthy" soil.

Fertilizer experiments were conducted in the greenhouse in soil which had been collected during the summer from diseased and healthy areas respectively, in infested fields. The samples were air dried as soon as possible after collecting. This was followed by a thorough mixing of the "diseased" samples to form one composite and of the "healthy" samples to form another, the two lots being then stored in this condition until used for experimentation two or three months later.

Preliminary experiments on wheat seedlings up to about six weeks, the stage at which browning symptoms are most striking in the field, showed that potassic fertilizers produced no differences on growth when applied either to diseased or to healthy soil; consequently no further tests with potassic fertilizers were considered necessary. Subsequent experiments were on the effects of phosphatic and nitrogenous fertilizers mainly.

Fertilizer Applications

All phosphatic fertilizers were applied at seed level at the rate of 1 gm. in Expts. 1 to 3, and 0.5 gm. in subsequent experiments, to each six-inch crock or pot at the time of sowing. Sodium nitrate was applied in solution at the rate of 1.5 gm. to each six-inch crock in the earlier and 0.75 gm. in the later experiments. Calcium nitrate was applied in amounts containing equivalent quantities of nitrate to the sodium nitrate application. The solution aliquots were applied at intervals, the first at the time of sowing, the second after two weeks, and the remaining portion after four weeks. The relative position of each crock on the greenhouse bench was changed at frequent intervals. Marquis spring wheat was used throughout the investigation.

Experiment 1. This experiment was conducted in duplicate. The plants were watered every second or third day with approximately the same amount of distilled water. Sowings were made on October 30, 1932, and the plants harvested 54 days afterwards (Fig. 5).

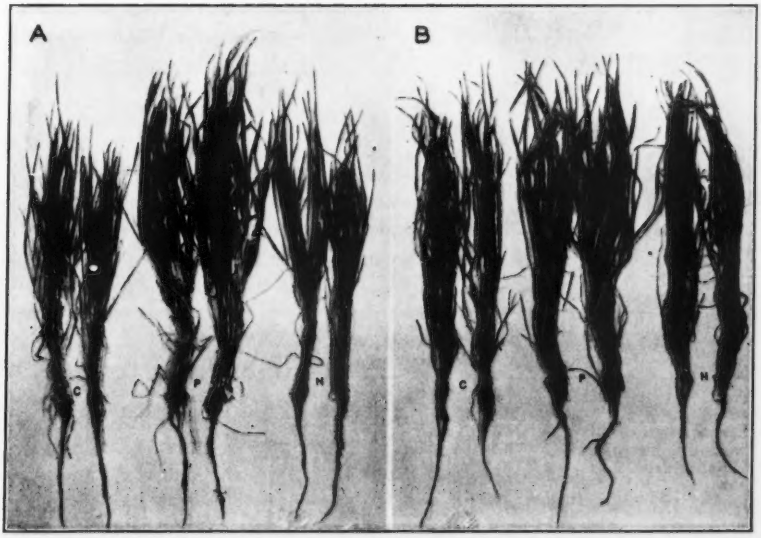

FIG. 5. *Showing response of wheat on* A, *browning soil, and* B, *normal soil, to phosphatic and nitrogenous fertilizers in a duplicate pot experiment; ten plants to each bundle.* C, *untreated control;* P, *receiving triple superphosphate, and* N, *receiving sodium nitrate. See Expt. 1, text and Table X.*

The results of Expt. 1 (Table X) show that in the untreated healthy soil there is approximately 50% increase in the oven-dried weight of the seedlings over those in the untreated diseased soil. In diseased soil the triple superphosphate dressing gave an increase of approximately 125%, while the nitrate fertilizers had no appreciable effect. In the healthy soil the phosphate dress-

ing gave an increase of approximately 33%, and the nitrate fertilizers again had no appreciable effect. This increased response to phosphate of diseased soil compared with healthy was not unexpected, since the diseased soil to begin with contained only about half as much available phosphorus as the

TABLE X

RESPONSE OF WHEAT ON SOIL FROM DISEASED AND HEALTHY AREAS TO VARIOUS FERTILIZER TREATMENTS

Experiment 1—54 days

Treatment	"Diseased" soil (mixture)			"Healthy" soil (mixture)		
	No. of plants	Average height, cm.	Oven-dry weight, gm.	No. of plants	Average height, cm.	Oven-dry weight, gm.
None—control	20	40	2.086	20	45	3.108
Triple superphosphate	19	50.7	4.701	20	47.7	4.283
Sodium nitrate	20	41.5	2.103	20	44.5	2.993
Calcium nitrate	20	42	2.238	19	44.5	2.881

Experiment 2—54 days

	"Diseased" soil* (single field)			"Healthy" burned straw-bottom soil†		
None—control	10	45	1.304	10	54	2.415
Triple superphosphate	10	46	1.685	10	50	2.325
Sodium nitrate	10	40	1.024	10	50	2.755
Calcium nitrate	10	41.5	1.098	10	53	2.509
Ashes	10	43	1.642			

Experiment 3—45 days

	"Diseased" soil (mixture)					
None—control	20	40	2.431			
Triple superphosphate	18	47	5.332			
Ammonium phosphate	20	44.5	6.393			
Sodium nitrate	20	41.5	2.709			
Straw	20	43.5	3.349			
Ashes	20	46.0	4.794			

Experiment 4—60 days

	"Diseased" soil (mixture)			"Healthy" soil (mixture)		
None—control	30	45	3.209	30	45	4.852
Triple superphosphate	30	46	7.139	30	48	7.248
Sodium nitrate	30	47	3.941	29	45	4.411

**Originally contained 10.6 p.p.m. nitrate nitrogen and 38 p.p.m. available phosphorus.*
†Originally contained 2.3 p.p.m. nitrate nitrogen and 129 p.p.m. available phosphorus.

healthy soil. It, however, contained about twice as much nitrate. The lack of response of the healthy soil to nitrate applications indicates that nitrate was not in this case a limiting factor though it was originally present in less amounts than in diseased soil. The possibility for favorable conditions for nitrification in this pot-culture work under greenhouse conditions must not be overlooked.

Experiment 2. This was conducted in one series on soil obtained from a single badly infested field, but otherwise in a manner similar to Expt. 1. The ashes were obtained from burned wheat straw and chaff and were applied at the rate of 5 gm. per crock. The determinations made on the samples collected in June showed that the diseased areas contained 38 p.p.m. available phosphorus and 10.6 p.p.m. nitrate nitrogen, while the healthy areas, where old straw stacks had been burned some two years previously, contained 129 p.p.m. available phosphorus and 2.3 p.p.m. nitrate nitrogen. The wheat plants on the healthy areas averaged 10 to 15 cm. taller than adjoining diseased wheat at harvest, and were 10 to 14 days earlier in maturing.

The results of Expt. 2 (Table X) showed that on the diseased soil, nitrate dressings caused a moderate decrease, while both phosphate and ashes caused a moderate increase over the untreated controls; on the healthy soil, nitrate produced a slight increase and phosphate a slight decrease. The original available phosphorus and nitrate contents of the soil samples probably offer a satisfactory explanation.

Experiment 3. Tap water was used for watering the plants in this and subsequent experiments as it was found that it contained negligible quantities of phosphate and nitrate. The straw amendment consisted of a small unmeasured amount of finely chopped straw which was thoroughly incorporated with the top three inches of soil in the two crocks a few days previous to sowing. Plate I, Fig. 1, shows the plants when harvested. Each bundle consists of two pot replicates of ten plants each.

From the data of Expt. 3 (Table X), it will be noticed that the phosphate applications again gave well over 100% increase in dry weight over the untreated control. A slight increase from nitrate is noticed. The straw, however, has given a substantial increase, while the ashes almost doubled the yield. The beneficial effect of the ashes as shown in Expts. 2 and 3 is probably due more to the phosphate than to the potash content, since, as previously mentioned, wheat on the open prairie soils was not benefited by potassic fertilizers.

Experiment 4. This experiment was conducted in triplicate in composite soil samples collected from 44 fields in 1933. Sowings were made on October 16, and the plants harvested 60 days afterwards.

These results are, in the main, in general agreement with those of Expt. 1 which was conducted on composite samples collected the previous year.

The data from Table X must also be viewed from the standpoint of increased or decreased resistance or liability of the host plant to fungal invasion. Plants receiving phosphate amendments or ashes showed no above-

ground symptoms of browning, but the nitrate-treated pots had as many discolored leaves as the controls. Pots with straw varied in this regard, depending presumably upon the degree of decomposition of the straw and its effect on the nitrate content of the soil. Typical *Pythium* root-tip lesions were found on the phosphate-treated plants, but the root systems as a whole had a decidedly white appearance while those of the nitrate-treated plants and the controls were quite yellow in contrast owing to the invasion of more fine laterals and cortical tissue by the fungus.

Experiments 5 and 6. Attempts were made in these experiments (Table XI) to determine the effects of various fertilizer dressings on the development and number of diseased crown roots, in diseased and healthy soil respectively.

TABLE XI

EFFECT OF SOIL AMENDMENTS ON THE DISEASE, WITH SPECIAL EMPHASIS ON CROWN-ROOT DEVELOPMENT

Treatment	No. of plants	Height, cm.	Oven-dry weight, gm.	Crown roots				
				Total no.	Length, cm.	% Healthy	% Diseased	H/D ratio
Experiment 5—41 days								
"Diseased" soil:								
None—control	30	37	4.854	66	—	44.0	56.0	0.8
Triple superphosphate	30	42	9.104	135	—	64.5	35.5	1.8
Sodium nitrate	30	36	4.986	66	—	27.2	72.8	0.37
"Healthy" soil:								
None—control	30	39	6.339	102	—	46.1	53.9	0.85
Triple superphosphate	30	39.5	8.356	131	—	66.4	33.6	1.9
Sodium nitrate	30	39	7.704	109	—	58.7	41.3	1.4
Experiment 6								
Soil I—"diseased":								
None—control	30	34	3.127	90	269	56.6	43.4	1.31
Triple superphosphate	30	37	5.595	135	695	60.7	39.3	1.56
Ammonium phosphate	30	38	5.572	133	568	63.1	36.9	1.71
Ammonium sulphate	30	36	3.632	83	143	57.8	42.2	1.37
Manure—12 tons per acre	30	38	5.554	151	515	45.7	54.3	0.84
Soil II—"diseased":								
None—control	28	—	2.880	93	—	9.7	90.3	0.11
Manure—12 tons per acre	28	—	4.540	177	—	31.6	68.4	0.46

The seeds were sown 2.5 in. deep to permit the development of a long sub-crown internode, which facilitated the separation of seminal and crown roots after careful and thorough washing in running water at the time of harvesting. No satisfactory methods of obtaining a disease rating of plants affected with *Pythium* root rot had been worked out, principally because of the difficulty of estimating the diseased condition of the very fine lateral roots which may show no sign of a diseased condition to the unaided eye, but which the microscope reveals to be filled with *Pythium* mycelium and generally some oospores as well. This destruction of the fine laterals is important since browning root-rot symptoms appear in some fields before the crown roots have developed. In such instances no severe lesioning may exist on the main primary roots, though the whole of the primary root system is yellower in mass than that of a healthy plant.

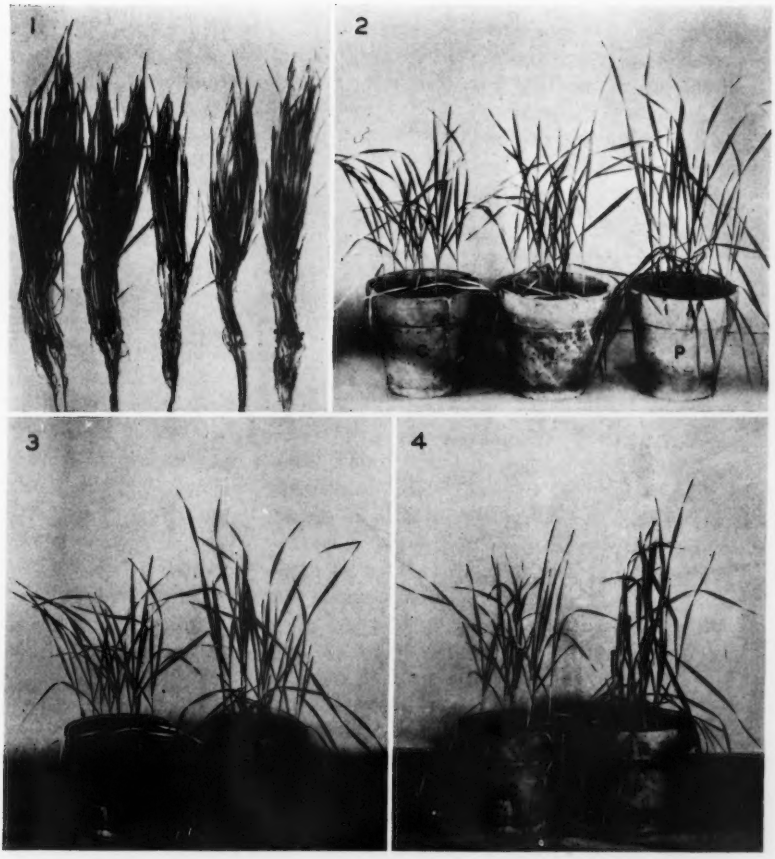

FIG. 1. *Six weeks old wheat seedlings in browning soil receiving the following treatments, left to right: triple superphosphate, ammonium phosphate, untreated control, sodium nitrate, and straw. See Expt. 3, text and Table X.* FIGS. 2 to 4. *Plants from Expt. 5, see text and Table XI;* C, *untreated browning soil,* N, *receiving sodium nitrate, and* P, *receiving triple superphosphate.* FIG. 2 *shows the inappreciable effect of the nitrate application and the beneficial effect of the phosphate application.* FIG. 3 *shows the typical yellow first foliage leaves of the diseased plants in the untreated browning soil as compared with the darker green vigorous plants in the phosphated plot.* FIG. 4 *shows the plants in* FIG. 3 *as they appeared two days later. Note that the second foliage leaves in the untreated plot are now yellow, while the first foliage leaves are yellowish brown and drier, and that the phosphated plants are unchanged.*

In Plate I, Figs. 2 to 4, are shown representative pots of Expt. 5 at the time the check plants showed typical browning symptoms. The effects of the fertilizers on the dry weights of these plants (Table XI) are of the same order as in Expts. 1 and 3. The effects on the crown-root systems are quite marked. In Expt. 5, in the diseased soil, the triple superphosphate has more than doubled the number of crown roots, while the sodium nitrate has had no effect; in the healthy soil the phosphate has increased the number by a little over one-quarter, while the nitrate has given no significant increase. Although the phosphate-treated plants in diseased soil showed no disease symptoms above ground (Plate I, Fig. 2, P), yet the increase in the number of crown roots is accompanied by an increase in the number of diseased roots. The higher healthy/diseased (H/D) ratio of these plants and the lower H/D ratio of the nitrate-treated plants over the control, suggest that the phosphate does tend to inhibit the disease, whereas the nitrate tends to augment it. The results in the healthy soil are what might be expected when the original available phosphorus and nitrate nitrogen content are borne in mind.

The outstanding results of Expt. 6 are that the phosphatic and manurial dressings have considerably increased the number and length of crown roots, and that this increase is associated with increased dry weights and greener plants. The phosphated plants usually have a slightly higher, and the manured plants a lower, H/D ratio of crown roots. It is characteristic of the manured plants that the large increase in the numbers of crown roots is concomitant with a large increase in the number of diseased roots; this results in the striking brownish yellow appearance of the roots in mass, which is usually the condition of check plants in diseased soil.

Nitrogenous fertilizers have produced no appreciable effect on crown-root development and consequently no difference in the growth of the plants in diseased soil.

At present it is believed that the H/D ratio of crown roots does not, alone, give an accurate disease index or rating when compared with the above-ground symptoms and dry weights. This will be made clearer by observing the dry weights and H/D ratios of the control and nitrate-treated plants in the diseased soil in Table XI. Similarly, the control in the healthy soil might be expected to have a much higher H/D ratio when compared with the control in the diseased soil. In the meantime, the H/D ratio should be considered only as one of the guides in estimating a disease rating.

It appears from the evidence that the beneficial effects of the phosphate applications and other treatments which widen the P/N ratio, are due primarily to the encouragement of a more vigorous root system and possibly only secondarily to increased resistance of the roots to fungal invasion. A detailed histological and microchemical study of the latter phase is a problem for further research.

Wheat following fallow on these soils does not respond to nitrate applications. This can be explained quite apart from the presence of any soil parasite. Nevertheless, in the browning root-rot problem there are indications

that relatively high nitrate associated with relatively low available phosphorus under wheat following fallow is another factor which renders the wheat plants more liable to fungal attack, since where the nitrate is reduced, as in the stubble crop on the same field say, the disease is ordinarily not manifest (*cf.* 46).

Since the application of phosphate to browning soil produces vigorous plants lacking aboveground "browning" symptoms, and increases the yield of grain appreciably, we feel justified in recommending the application of phosphate fertilizers as a control measure for *Pythium* root rot of cereals, even though the question whether the plants are made more disease-escaping or more resistant still remains to be worked out.

The Relation of *Pythium* to the Disease

The results reported in the foregoing sections indicate that unbalanced soil nutrients play an important role in the root-rot complex. The following comparative experiments were planned to determine whether the disease is solely one of mineral deficiency or unbalanced metabolism or is caused primarily by the fungal parasite. In this work no attempt has been made to determine whether the various sterilization treatments produced a stimulating effect on plant growth. It is recognized that total or partial sterilization of soil by steam or by chemicals will produce a stimulation in plant growth due mainly to an increase in available nutrients, and partly to a change in the biological equilibrium of the soil microflora. Waksman (47) has pointed out that "there is no method available for sterilizing the soil without changing its physical and chemical properties". However, Cooke (11), working with Hawaiian soils, found that chloropicrin produced "no purely stimulating effect". The limitations of the soil sterilization method are recognized.

Experiments 7 and 8. In these experiments (Table XII), the respective soil sterilization treatments were carried out in six-inch pots in duplicate 14 days before sowing. The formalin was applied at the rate of $\frac{1}{2}$ gallon of a 2% solution per square foot, and the chloropicrin at 200 lb. per acre. The steam sterilized soil was subjected to 15 lb. pressure for 2 hr. In the preliminary trial (Expt. 7), the formalin approximately doubled the yield, while the phosphate gave about 85% increase. In Expt. 8, the average increase in dry weight for the three sterilization treatments was approximately 80% in the diseased soil and 40% in the healthy, when compared with their respective controls. The healthy soil has given an increase over the diseased soil that is greater than usual for healthy soils. The average dry weight of the three sterilization treatments is the same, namely, 3.925 gm. in the diseased soil and 3.931 gm. in the healthy, though the yield of the diseased control is considerably lower than that of the healthy. It does not seem justifiable, therefore, to base the results of these two sterilization series on chemical stimulation alone, but rather some benefit should be attributed to the removal of the parasitic fungi by sterilization also. Incidentally it appears that steam sterilization has inhibited crown-root development.

TABLE XII

A COMPARISON OF THE EFFECTS OF PHOSPHATE APPLICATION AND STERILIZATION OF BROWNING SOIL IN RELATION TO THE GROWTH OF MARQUIS WHEAT

Treatment	No. of plants	Oven-dry weight, gm.	Crown roots			
			Total no.	% healthy	% diseased	H/D ratio
Experiment 7						
"Diseased" soil:						
Spring, 1934—6 weeks						
None	20	2.434				
Superphosphate	20	4.514				
Formalin	20	4.834				
Experiment 8						
"Diseased" soil:						
Autumn, 1934—7 weeks						
None	20	2.138	9	66.6	33.3	2.00
Superphosphate	20	3.102	38	68.4	31.6	2.17
Formalin	20	3.684	26	100	0.0	
Chloropicrin	20	4.224	25	100	0.0	
Steam	20	3.869	2	100	0.0	
"Healthy" soil:						
None	20	2.828	18	66.6	33.3	2.00
Superphosphate	20	3.332	21	57.1	42.8	1.33
Formalin	20	3.820	20	100	0.0	
Chloropicrin	20	4.428	18	100	0.0	
Steam	20	3.546	3	100	0.0	

In an experiment conducted on drifted top soil and wind eroded soil (subsoil), it was found that the total number of *Pythium*-infested crown roots was greater in the top soil than in the eroded soil, in untreated, phosphated and manured pairs. The untreated top soil gave a better yield than the untreated eroded soil, but the phosphated and manured eroded soil gave actual higher yields of plant growth than the corresponding pairs in top soil. This suggests the probable greater activity of the parasite in the surface soil.

Dickson (13) holds that "the parasite rightly is considered the primary cause of the parasitic disease in plants" when considered in relation to the conditioning or predisposing factors of the environment. From our earlier work on parasitism (46), as well as the results reported here, it appears that species of *Pythium* are the primary cause of browning root rot of wheat, but that unbalanced nutrients obtaining under the summerfallow crop on certain phosphorus deficient soils of Saskatchewan, predispose the seedlings to attack. The effect of the unbalanced nutrients on the parasitic vigor of the fungus is still an unsettled problem.

V. PRELIMINARY PLANT ANALYSES

It has already been pointed out that plants absorb nutrient ions in approximately the proportions in which these ions are present in the nutrient medium (9, 10, 38, 39, 43). Pearsall and Ewing (33), working with radish and turnip principally, found that with abundant nitrates in the external medium there

was little protein and relatively high amounts of soluble nitrogen in the plants and, conversely, with low nitrates in the medium there was higher protein nitrogen and lower soluble nitrogen. They state that "An abundant supply of nitrates leads normally to the accumulation of amino-acids, which are formed more rapidly than they are used". It became necessary, therefore, to determine the phosphorus and nitrogen fractions in diseased and healthy plants and compare the results with the available phosphorus and nitrate nitrogen content of the soil areas on which such plants were grown.

The greenhouse material, consisting of both tops and thoroughly washed roots, was oven-dried at 100° C. immediately after harvesting, while in the case of the field material, which consisted of tops only, some sample pairs were dried on the day of collecting and others on the following day, depending on the distance of the field from the laboratory. This method probably had no effect on the phosphorus content, but may have changed appreciably the nitrogen fractions. With the nitrogen determinations, therefore, the greenhouse material should be taken as more indicative of the actual fractions in the growing plants. The total phosphorus, and the total and water-soluble nitrogen were determined by prescribed A.O.A.C. methods (32).* The protein nitrogen was obtained by difference. The phosphorus results are presented in Table XIII, and the nitrogen results in Table XIV.

TABLE XIII

PERCENTAGES OF TOTAL PHOSPHORUS IN DISEASED AND HEALTHY PLANTS, JUNE, 1934

Field	Locality	Phosphorus, %		Diseased as a percentage of healthy
		Diseased	Healthy	
1	(Composite sample)	0.136	0.238	57.1
2	Adanac	0.170	0.273	62.3
3	Paynton	0.155	0.368	42.1
4	Maymont	0.165	0.400	41.2
5	Lac Vert	0.163	0.248	65.7
6	Humboldt	0.139	0.231	60.2
7	Dana	0.153	0.198	77.3
8	Plunkett	0.146	0.226	64.6
9	McLean	0.140	0.267	52.4
10	(Composite sample)	0.154	0.198	77.8
11	(Composite sample)	0.145	0.311	46.6
12	(Composite sample)	0.158	0.292	54.1
	Mean	0.152	0.271	58.45

Of the twelve pairs analyzed (Table XIII), the healthy sample in every case contained a higher percentage of total phosphorus than the diseased sample. The mean of the total phosphorus content of diseased plants considered as a percentage of the healthy is 58.45, which is roughly of the same order as the available phosphorus content of diseased soil considered as a percentage of healthy soil (*cf.* Mean, 1932-33, Table II).

**My thanks are due to J. S. Clayton, M.Sc., of the Cereal Chemistry Laboratory, for making these chemical determinations.*

TABLE XIV

PERCENTAGES OF NITROGEN IN DISEASED AND HEALTHY PLANTS, JUNE, 1934

Field No.	Locality	Nitrogen							
		Diseased				Healthy			
		Total, %	Protein, %	Water-soluble, %	Ratio, P.N./W.S.N.	Total, %	Protein, %	Water-soluble, %	Ratio, P.N./W.S.N.
Greenhouse:									
	Composite	3.54	1.40	2.14	0.65	4.37	2.28	2.09	1.09
	Composite	3.86	1.06	2.80	0.37	4.54	1.63	2.91	0.56
	Mean	3.70	1.23	2.47	0.51	4.45	1.95	2.50	0.82
Field:									
1	(Composite sample)	4.26	2.86	1.40	2.04	4.83	3.64	1.20	3.04
2	Adanac	4.12	2.78	1.34	2.07	4.22	2.96	1.26	2.34
3	Paynton	4.09	2.34	1.75	1.34	4.79	2.78	2.01	1.38
4	Maymont	4.22	2.08	2.14	0.97	5.14	2.79	2.35	1.18
5	Lac Vert	3.58	2.14	1.44	1.47	3.81	2.16	1.66	1.30
6	Humboldt	3.01	1.26	1.75	0.72	3.30	1.77	1.53	1.15
7	Dana	3.31	1.73	1.57	1.10	3.12	1.54	1.57	0.98
8	Plunkett	3.03	2.02	1.01	2.00	3.49	2.19	1.29	1.70
9	McLean	3.36	2.30	1.05	2.18	4.16	2.87	1.28	2.24
10	(Composite sample)	2.62	1.82	0.80	2.28	2.93	1.68	1.25	1.34
11	(Composite sample)	3.56	2.42	1.14	2.12	4.45	3.45	0.99	3.47
12	(Composite sample)	4.01	2.25	1.77	1.27	4.55	2.78	1.77	1.57
	Mean	3.60	2.17	1.43	1.64	4.07	2.55	1.51	1.81

The figures of most interest in Table XIV are the protein nitrogen and water-soluble nitrogen ratios which are lower in the diseased soil than in the healthy in both the greenhouse and field material. That is, there is relatively *more* water-soluble nitrogen and relatively *less* protein nitrogen in diseased than in healthy plants. While these results are decidedly preliminary, they are in general agreement with the findings of Pearsall and Ewing (33). However, it is possible that as more and more of the roots in the diseased areas become infected, normal intake and subsequent utilization of the various nutrients will be greatly disturbed. This may in part account for the generally higher percentage of total nitrogen in healthy plants, though the nitrate nitrogen content of healthy soil is usually lower than that of diseased soil.

Rapid chemical tests for phosphate and nitrate. Wheat seedlings grown in the greenhouse in composite samples of browning and of normal soil collected in early June, 1934, were tested for phosphate and nitrate when the seedlings in the browning soil showed disease symptoms. The methods used were the semi-quantitative rapid chemical tests on plants as described by Thornton and co-workers (44).

Stem portions of diseased seedlings from the browning soil showed a lower content of phosphate and a greater content of nitrate in the sap than healthy seedlings from normal soil. The same relation existed in the roots of diseased and healthy seedlings which were very thoroughly washed and dried between filter paper before testing. Similar results were obtained with maize seedlings. In some fertilizer experiments, applications of either phosphate or nitrate to the soil were readily detected by an increase of these constituents in the sap of the seedlings. The results obtained, though semi-quantitative, were clear cut.

If, as has been suggested (33), with abundant amino acids the cellulose walls become more swollen, and also, as some contend, with high phosphorus the cell walls become harder, then it is reasonable to assume that the unbalanced soil nutrients in certain soil areas lead to a condition in the plants which renders them more liable to the depredations of the parasite. At the same time it must be recognized that a particular balance of nutrients in one soil area may encourage a vigorous vegetative growth in the parasite, in which state it might be most parasitic, whereas in another area conditions may favor sporulation or a relatively quiescent state.

Chlorophyll Content

It is generally conceded that the higher the nitrate content of the soil the greener will be the plants growing thereon (33). Several comparisons were made of the chlorophyll extracts of the tops of healthy and diseased plants from which the lower discolored leaves had been removed. No consistent visible differences could be observed in the respective chlorophyll extracts. It is possible that the damaged root systems of the plants from the diseased areas may have prevented efficient absorption and subsequent utilization of the higher soil nitrate.

General Discussion

In *Pythium* root-rot districts the disease is principally confined to the wheat crop following bare fallow; the stubble crop is rarely sufficiently attacked for aboveground symptoms to appear. The chemical analyses reported in this paper have shown that available phosphorus in the soil is lower and nitrate nitrogen higher in browning areas than in normal areas. It is necessary, therefore, to consider the soil and crop-culture factors, especially the practice of summerfallowing, as they exist in north-central Saskatchewan. The majority of prairie soils, though potentially rich in nutrient elements, respond to certain fertilizer applications (14). Recently a deficiency of available phosphorus in many of these soils has become manifest (14, 29). The replenishment of the nitrate removed by the crops is accomplished by the practice of bare summerfallow cultivation, but the amount of nitrification is closely dependent on seasonal conditions and on frequency of cultivation. In browning root-rot localities and in fertilizer pot trials, no increase in yield of cereals as a result of potash applications has been observed. Potassium is therefore considered to be present in sufficient quantities in Saskatchewan

soils where *Pythium* root rot occurs. Wyatt and Newton (51), however, obtained beneficial results from applications of potash on the gray, wooded soils of northern and western Alberta and sometimes also on the black soils of Alberta. The majority of Saskatchewan soils are neutral or slightly alkaline (Tables I and II) with some podsols showing slight acidity, but no evidence of iron or manganese deficiency has been demonstrated as yet. The commoner crop rotations on the prairies are wheat-wheat-summerfallow or wheat-oats-summerfallow, as the majority of farms are devoted almost entirely to grain growing. Mixed farming with grain growing still of major importance is found in the park belt to the north and northeast. This continuous system of grain growing in many of the older districts or on poorer soil in newer districts has apparently reduced the available phosphorus to an amount insufficient for the needs of the crop. Until recently, fertilizer amendments were never applied to replenish the essential elements removed by the crop, and owing to the type of farming, comparatively little farmyard manure has been applied to the land. The foregoing facts, therefore, doubtless account for the low available phosphorus in browning districts, but it is also possible that some of these soils are naturally highly phosphate-fixing, as indicated by the work of Cooke (11), Carpenter (8) and others working on *Pythium* root rot of sugar cane in Hawaii.

The chief purpose of bare summerfallowing on the prairies is to conserve soil moisture for the following crop. Nitrate nitrogen increases because the conditions favor rapid nitrification, and weeds are kept in check by the intermittent cultivation. The lack of positive response of wheat following fallow to nitrogenous fertilizers indicates that the open prairie soils can still produce enough nitrate for the needs of the crop. It should be pointed out, however, that the gray, wooded soils of northern Alberta (51) and northern Manitoba (14) and very likely of northern Saskatchewan also, produce definite increases in yield from nitrogenous fertilizers. Barnes (1) has questioned whether a nitrogen problem really exists in the extensive dry-land farming areas of Western Canada. The moisture hazard appears to be the dominant problem. Now it is possible that in the north-central and northeastern districts where precipitation is usually sufficient for the needs of the crop, excessive cultivation of the summerfallow, performed purposely to control weeds and not necessarily to conserve moisture, may permit the accumulation of nitrate in excess of the demands of the seedlings. They may absorb more nitrate than can be elaborated into reserve nitrogen compounds in a given time. The nitrate may not be unduly excessive, but it may be above the critical level for that element. This condition, if associated with an insufficiency of available phosphorus, may well upset the metabolism of the wheat seedlings at the critical stage of crown-root development and the beginning of the tiller formation. Now well-tilled fallow may contain considerably more nitrate than poor fallow (23); this fact may be of significance in explaining why *Pythium* root rot of wheat is frequently most severe on the best fallows in a browning district. The second or stubble crop of wheat following a browning-diseased crop on summerfallow, does not ordinarily

develop browning symptoms. In such a case the nitrate is considerably lower than in the fallow crop, while the available phosphorus has remained practically the same according to available evidence (29). The disease may be prevented from becoming manifest by (*i*) reducing the amount of nitrate nitrogen of many excessively cultivated summerfallow fields in browning districts, as occurs naturally when a stubble crop is grown; and (*ii*) by increasing the amount of available phosphorus by the application of phosphatic fertilizers at the time of sowing the summerfallow crop. Thus it appears that there is a correlation between available phosphorus and nitrate nitrogen and disease expression. It seems also that either "balanced" deficiencies or balanced excesses will inhibit root rot of wheat. Experiments with sugar cane (8, p. 332) indicated that balanced deficiencies did not lead to root rot, but the question of balanced excesses has not been settled.

It is well known that the addition of strawy organic matter to the soil decreases, temporarily, the available nitrate, and our experiments have demonstrated that the addition of cereal straw to browning soil tends to inhibit the disease. This may be due to increased biological antagonism. It is possible, however, that the carbon dioxide evolved by the decomposing organic matter increases the availability of phosphorus. Thus, in Louisiana, both Meyer (28) and Sturgis (42) have shown the importance of increasing or maintaining the availability of phosphorus by the addition of trash to the soil. Meyer considered that as the organic matter is depleted, the soil phosphates as well as those added become less effective. Sturgis found that the increase was most marked in the earlier stages of decomposition. Pittman and Burnham (35) pointed out that in Utah, manuring brings much of the unavailable soil phosphate into action. Now the ploughing under of weeds or other organic matter invariably produces better growth in browning fields. It might reasonably be postulated that the biological activities have reduced the soil nitrate and increased the availability of the soil phosphorus. This may also explain the normal or healthy areas in otherwise browning-diseased fields. The changes in the P/N ratio in the soil under the prevailing crop-culture methods are now more understandable. Our results indicate that an increase in the P/N ratio is accompanied by an inhibition of *Pythium* root rot, while a decrease promotes the disease. The available phosphorus and nitrate nitrogen ratios as shown in Table III, suggest that low available phosphorus is more closely correlated with the disease than high nitrate nitrogen, that is, if the two can be considered separately.

The experimental results of the effects of phosphatic and nitrogenous dressings on *Pythium* root rot in browning and normal soil are in general agreement with the expectations based on the chemical analyses of the soils. Three years' observations of phosphate applications to wheat on fallow in browning districts have shown that the fertilizer has inhibited the root rot generally as indicated by the uniform growth and the absence of patches of brown, dwarfed plants so characteristic of unfertilized check strips. The evenness

of the phosphated fields persists until maturity, resulting in uniformity of ripening and plumpness of grain. The effects of various amendments on the crown-root development in browning and normal soil are very illuminating (Table XI). Phosphates and farmyard manure frequently double both the number and the total length of crown roots of wheat in browning soil. The ratio of healthy to diseased roots is not always increased thereby, but "normal" green plants develop (Plate I, Fig. 3, P); this suggests that the more vigorous root systems have allowed the plants to become disease-escaping rather than more resistant. This phase requires further elucidation. Nitrate applications to browning soil usually have no effect on crown-root development; sometimes, however, there is a decrease in number and length as well as a decrease in the healthy/diseased ratio. Our present knowledge of the effects of the soil differences on the parasitic vigor of the fungus is very meagre, and could be discussed only speculatively.

It must not be inferred from the results of the chemical soil analyses and fertilizer experiments that the disease is due merely to nutritional disturbances. In a previous communication (46) it was shown that *Pythium* spp., principally *P. arrhenomanes*, are constantly associated with the lesioned roots of browning seedlings and that they can be readily isolated and made to reproduce the disease artificially. The Canadian strain of *P. arrhenomanes* is vigorously parasitic on wheat seedlings. In this paper some evidence is presented which suggests that the parasite does play an active part.

The plant analyses for total phosphorus and total and water-soluble nitrogen submitted in this paper are only preliminary, although they are in general agreement with the work of others on percentages of phosphorus and nitrogen in plants relative to the amount of these elements in the external medium. The main points ascertained were: firstly, that the total phosphorus was *lower* in diseased than in healthy plants; and secondly, that there is relatively *more* water-soluble nitrogen and relatively *less* protein nitrogen in diseased than in healthy plants. The semi-quantitative rapid chemical tests on diseased and healthy seedlings grown in browning and normal soil in the greenhouse showed a lower content of phosphate and a higher content of nitrate in the sap of diseased than of healthy seedlings. Under the more or less uniform conditions in the greenhouse, no differences in resistance to *Pythium* root rot of the commonly grown wheat varieties have been brought out. Under the more extreme conditions of the field slight varietal differences have shown up in some years, but further field observations are necessary before a definite pronouncement can be made.

The marked similarity between *Pythium* root rot of wheat and *Pythium* root rot of sugar cane is very striking and calls for special comment. Carpenter's recent paper (8), which has just come to hand, presents a capable review of the important findings on the cane disease during the last four years. It appears from the comparative culture studies of Rands and Dopp (36) that the causal organisms in both diseases are geographic strains of *P. arrhenomanes* Drechs. In his earlier work, Carpenter (6, 7) pointed out

that excess nitrogenous fertilizers promoted the cane root rot and that the disease incidence varied with the nutrition. More recently, Cooke (11) demonstrated that low available phosphorus on many of the phosphate-fixing soils predisposed the cane to fungal invasion. Heck (17) shortly afterwards considered that *Pythium* root rot of cane was "induced by an unbalanced nutrition in which low phosphorus and high mineral nitrogen are more or less outstanding". This is in agreement with our findings of 1932 and 1933 on *Pythium* root rot of wheat which were reported locally and are published here for the first time. It would appear from Carpenter's last contribution (8) that the phosphate-nitrogen ratio is one of the chief lines of investigation on the cane root rot at present; other unfavorable balances of nutrients and deficiencies of other elements are also being given considerable attention. He pointed out that some varieties of cane are made more susceptible with nitrogen in excess, while other varieties not particularly sensitive to increased nitrogen fall a prey to the parasite under deficiencies of available phosphorus.

In conclusion, our present knowledge of soil factors influencing *Pythium* root rot of wheat indicates that unbalanced available phosphorus and nitrate nitrogen, or perhaps in some instances low available phosphorus alone, is most likely concerned in predisposing the seedlings or rendering them liable to fungal attack.

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A MICROBIOLOGICAL STUDY OF PODSOL SOIL PROFILES

II. LAURENTIAN SOILS[1]

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Abstract

Microbiological studies of samples from the separate horizons of two soils from the Laurentian uplands showed that, as in the case of the soils previously studied from the Appalachian uplands, biological activity was dependent upon the organic-matter relations of the horizons. A_1 (organic-matter) horizons were the most active, as shown by the evolution of carbon dioxide, nitrification of the soil's own nitrogen, by numbers of micro-organisms (bacteria, actinomyces, and fungi) and by the production of ammonia from urea. Bacterial numbers in the illuvial horizons were, however, higher than in the eluvial (leached) horizons above, a condition that did not obtain with the Appalachian soils. The organic matter of a soil bearing a coniferous (spruce) flora was deficient in nitrifying power. The biological activity at different levels of a virgin clay soil was found to be in sharp contrast with that of the podsol soils.

In a recent paper the characteristics of the separate horizons of three heavily leached podsol soils from the Appalachian uplands of Quebec were described (3). The upland podsol soils of the Laurentian plateau are different in several respects from those of the Appalachian chain; their dissimilarity is due mainly to the nature of the parent rock, and less to the climatic environment. A study of two such soils revealed the fact that they contained much more calcium, and more magnesium and potassium than the Appalachian soils, in both the surface soil and the subsoil (6). This suggested that biological activity in horizons of the Laurentian soils might prove to be considerably greater than in horizons of the Appalachian, at least in regard to such activities as have been shown to be to some extent dependent on basic constituents.

This paper is intended to serve as an introduction to the biological characteristics of the separate horizons of podsol soils situated within agricultural districts in the Laurentian plateau, in the Province of Quebec. One soil possessed a cover chiefly of deciduous trees; this soil was selected as reasonably conforming in character with those previously studied from the Appalachian system. A second soil from under a stand of coniferous trees was selected to enable a comparison to be made with the horizons from soil under a stand of deciduous trees, since it has been claimed that the bio-dynamics of such coniferous soils are distinctive, in that nitrification may be adversely

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affected by an excess of resins, terpenes, and similar compounds, (4, 5); it has also recently been found that a spruce raw humus may be inactive in regard to nitrification (1).

TABLE I

DESCRIPTION OF SOIL PROFILES

Sample and date collected	Ecology	Depths of horizons sampled, and description
LM June 4, 1933	Mixed coppice of beech, birch, poplar, spruce; secondary growth. 800 ft. above mean sea-level. Lac Mercier, Labelle Co., Que.	A_1, 2 in. black, mucky, spongy, organic matter, sinking into leached layer below. A_2, 3 in. gray sandy loam, blackish at top from superficial horizon. B_1, 4 in. reddish-gray sand with gravel; infiltration from A_2 horizon; red streaks. B_2, 3 in. red gravelly sand, extending to below 12 in., where subsoil is richer in iron.
VC June 8, 1933	Regenerated spruce forest. 500 ft. above mean sea level. Val St. Michel, Quebec Co., Que.	(A_0, not collected; spruce needles, decomposing with gray fungus flora). A_1, 2 in. dark, fibrous, spongy, semi-decomposed organic matter, with fibrous root material. A_2, 4 in. gray sand. B, 6 in. red-brown sand, extending to below 12 in., with "ortstein" concretions fragmenting by hand pressure; organic matter and fibrous roots present.

In order to compare the biodynamics of a representative soil of the type that is not affected by climate to such an extent, samples were also taken from a virgin clay soil, at 3-inch intervals down to a depth of one foot. No chemical analyses were made with these subsamples. The separate horizons were sampled in the manner previously described (3).

The analytical methods used were those described in the previous study. In addition, analyses were made by the Department of Chemistry, Macdonald College, for certain physical and chemical factors, as shown in Tables II and III.

TABLE II

PHYSICAL CHARACTERISTICS OF SAMPLES FROM SOIL HORIZONS

Soil	Horizon	Moisture in fresh soil, %	Hygroscopic moisture, %	Loss on ignition, %	Water-holding capacity, % of air dried soil
LM	A_1	73.28	7.21	47.46	165.6
	A_2	16.81	0.28	1.60	26.0
	B_1	14.09	0.22	1.02	23.1
	B_2	19.02	0.63	2.40	24.9
VC	A_1	49.42	6.15	50.78	217.6
	A_2	20.36	0.23	1.44	31.5
	B	43.01	9.37	32.70	59.0

TABLE III

CHEMICAL CHARACTERISTICS OF SAMPLES FROM SOIL HORIZONS

Soil	Horizon	Organic carbon	Total nitrogen	Ratio C/N	CaO	MgO	P_2O_5
LM	A_1	22.65	1.018	22.25	1.43	0.46	0.13
	A_2	0.88	0.051	17.26	2.05	0.82	0.06
	B_1	0.36	0.027	13.36	2.19	0.90	0.12
	B_2	0.88	0.060	14.67	2.23	0.87	0.17
VC	A_1	25.47	0.861	29.58	0.78	0.26	0.14
	A_2	0.72	0.049	14.69	0.99	0.19	0.06
	B	9.80	0.167	58.68	0.91	0.35	0.12

The biological determinations made were those for the evolution of carbon dioxide, for the estimation of bacteria and actinomyces on Thornton's medium, and of fungi on a dextrose agar of pH 4.0, for nitrification of the soil's own nitrogen, and for the production of ammonia from urea. The results are shown in Tables IV to VI, and clearly indicate an activity similar in most respects to that found in the separate horizons of the Appalachian soils.

The biological differences between the organic matter horizons and the leached horizons were very similar to those in the Appalachian upland soils; the average proportional level of activity, as represented by the evolution of carbon dioxide, in the leached horizons was 3.7% of that in the organic matter horizons (Table IV). In regard to bacteria and actinomyces, a

TABLE IV

BIOLOGICAL CHARACTERISTICS OF SAMPLES FROM SOIL HORIZONS (ON BASIS OF OVEN DRIED SOIL)

Soil	Horizon	Carbon dioxide, mg. per 100 gm. per 100 hr.	Bacteria per gm.	Actinomyces per gm.	Fungi per gm.
LM	A_1	211.60	9,792,000	1,104,000	190,700
	A_2	7.85	369,000	53,300	10,280
	B_1	2.78	399,900	(1,000)	(1,000)
	B_2	4.52	1,006,000	(1,000)	(1,000)
VC	A_1	278.10	8,209,000	446,000	487,000
	A_2	14.06	550,000	30,830	2,690
	B	17.56	593,000	12,370	5,580

proportional value of 5.9% was obtained for both groups of organisms. It is of interest to note that the amounts of carbon and nitrogen in the leached horizons were, on the average, 3.4 and 5.0% respectively of the carbon and nitrogen in the organic matter horizons.

The numbers of bacteria in the illuvial horizons of both Laurentian profiles were slightly higher than they were in the leached horizons; this can be contrasted with the condition of the same horizons of the Appalachian soils. The same distinction cannot be made with regard to the actinomyces, for in all of the soils so far examined, their numbers were least in the illuvial horizons.

An outstanding difference will be noted in Table V, in respect of nitrification in the "coniferous" soil (VC); no nitrates were formed in the eluvial horizons A_1 and A_2, though nitrogen in these was not considerably less than in the corresponding horizons of the Laurentian soil with a "deciduous" cover.

TABLE V

NITRIFICATION IN SAMPLES FROM SOIL HORIZONS
(Nitrate nitrogen, parts per million, oven dried soil)

		5 days	51 days	104 days	317 days
LM	A_1	trace	275.10	453.90	599.20
	A_2	nil	10.78	19.11	27.60
	B_1	nil	nil	8.56	10.39
	B_2	nil	nil	9.95	10.18
			47 days	100 days	313 days
VC	A_1	—	nil	nil	nil
	A_2	—	nil	5.6	trace
	B	—	14.76	21.94	29.72

Reference to Table III will show that horizon B of soil VC, in which nitrates were formed to a greater extent than in any other horizon except A_1 of soil LM, contains a high proportion of organic carbon as well as nitrogen. From this it seems possible to conclude that there was less of any inhibitory substances such as may have been present in the two superficial horizons, or that the nitrogen compounds present in the illuvial horizon were different from those in the eluvial horizons. The organic matter present, as both carbon and nitrogen, clearly affected the moisture conditions in this lowest horizon, as shown in Tables I and II. The organic carbon clearly did not affect the evolution of carbon dioxide, nor were the numbers of micro-organisms dependent upon it, in comparison with the other horizons. The horizon under discussion contains, also, a lower percentage of bases and of phosphoric acid than those in which nitrification was not so well developed. In view of the prevalence of coniferous soils, it would appear necessary to make a closer study of the conditions that affect the activity of the nitrifying bacteria, since many such soils, after cultivation, prove to be relatively infertile and have a low capacity for nitrification (4).

TABLE VI

PRODUCTION OF AMMONIA FROM UREA, BY SAMPLES FROM SOIL HORIZONS
(Mg. NH_3 nitrogen per cc. of culture)

		24 hr.	53 hr.	72 hr.	120 hr.	168 hr.	11 days	20 days	40 days
A. *Deciduous podsol soil*									
LM	A_1	0.57	10.17	17.63	**23.29**	22.80	23.26	23.17	19.97
	A_2	0.20	2.27	3.84	7.69	9.84	12.66	**13.19**	12.46
	B_1	0.08	0.47	1.76	5.25	6.97	9.23	**11.75**	8.81
	B_2	0.05	0.97	2.02	4.73	7.72	9.82	**10.06**	8.88
B. *Coniferous podsol soil*									
VC	A_1	0.27	6.11	17.66	24.15	**25.14**	22.42	22.92	20.98
	A_2	0.13	1.01	2.17	5.59	8.14	11.16	**11.87**	10.62
	B	0.07	0.20	1.34	6.77	10.54	15.19	**16.12**	14.27

NOTE:—*To each 50 cc. of 5% urea solution, containing 0.05% K_2HPO_4, was added a mass of moist soil equivalent to 5 gm. of oven-dried soil.*

In the test for the decomposition of urea, the maximum amount of ammonia was produced by the organic-matter horizon of the "deciduous" soil after 5 days' incubation (See Table VI). In none of the other horizons was the limit of decomposition attained, even after 40 days' incubation. It is of interest to note that the illuvial horizon of the "coniferous" soil, having a high percentage of carbon, produced more ammonia than the eluvial (leached) horizon; in this experiment, however, as in that reported in the previous study, the ammonia produced was not proportional to the amount of organic carbon.

The results obtained from the separate layers of the clay soil show clearly the difference between the two types of soil. The values for moisture, evolution of carbon dioxide, and for bacteria are shown in Table VII.

TABLE VII

BIOLOGICAL ACTIVITY IN SAMPLES FROM DIFFERENT DEPTHS OF A VIRGIN CLAY SOIL

Depth of sample, in.	Moisture, %	Carbon dioxide mg. per 100 gm. per 100 hr.	Bacteria per gm. dry soil
0— 3	26.89	17.50	15,540,000
3— 6	20.98	8.55	7,404,000
6— 9	21.40	5.28	9,721,000
9—12	24.10	3.29	6,561,000

Conditions similar to those in the clay soil are probably prevalent in most soils in which the organic matter is evenly distributed vertically to some depth. Brown and Benton (2) report bacterial numbers of the horizons of several agricultural soils of Iowa; they include one virgin soil under grass, in which there was a sharp decline between horizons, but the decline occurred well below the usual limit of ploughing.

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A MICROBIOLOGICAL STUDY OF PODSOL SOIL PROFILES

III. BACTERIA FOUND IN SEPARATE HORIZONS[1]

By P. H. H. Gray[2]

Abstract

An investigation has been made to determine the presence of bacteria concerned in certain important biochemical reactions in the separate horizons of five heavily leached (podsol) soils of Quebec. Cellulose decomposing bacteria were represented by a species of *Micrococcus*, and by *Cytophaga hutchinsoni*. Organisms of the group *Mycobacteriaceae* were found in mediums devised to isolate bacteria able to decompose phenol and naphthalene. Of the nitrogen-fixing bacteria the aerobic organism *Azotobacter* was absent, but the anaerobic organism, *Bacillus amylobacter*, was present in all of the horizons. The decomposition of urea was due to Gram-negative spore-forming rods whose systematic position has not been determined.

Podsol soils owe their peculiar characteristics to the fact that under the climatic conditions in which they are most commonly found a large part of the organic residues in the surface horizons remains only partially decomposed. Such decomposition as occurs gives rise to considerable acidity, that removes from the mineral part of the soil below a large proportion of the available bases.

The biological problems presented by the well defined horizons of podsol soil profiles appear to have important bearings in relation to the ecological aspects of forestry and agriculture. The conditions that encourage a certain type of forest cover will affect the value of the soil in agriculture. This is due largely to the nature of the residues from the forest cover, and their ultimate decomposition or resistance to the action of soil micro-organisms. From the aspect of microbial nutrition with especial reference to sources of carbon, it would appear that the important fractions of the plant residues consist in (a) those soluble in water, (b) the more resistant materials, including cellulose, lignin, and protein compounds. Among the compounds commonly associated as lignin, or the lignin-humus complex, are phenolic and other compounds with a ring structure; similar compounds may be present in the protein fractions. These fractions have been found to differ according to the nature of the cover, but studies have not as yet been made to determine the specific sources of biological activity; it is therefore impossible at present to state that one group or another of micro-organisms is of the greater importance in the transformation of the residues.

A number of studies have been made on the nature of the biochemical activity of the undecomposed and semi-decomposed horizons of the organic

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mat overlying the soil, chiefly in relation to silviculture, and the presence of mycorrhiza and other fungi reported. The formation of ammonia and of nitrate nitrogen in forest soils has also received attention, and some studies have been made on the bacterial flora of forest soils under different climatic conditions. In regard to podsol soils, Rasumov and Remesov (4) have determined the morphology and number of organisms, by a direct method, in separate horizons of soil profiles. No studies have yet been made as to the nature of the bacterial flora concerned in processes of decomposition in the separate horizons of soils representative of the podsol group.

The present paper reports a preliminary survey of certain groups of bacteria, isolated from the separate horizons of a few heavily leached podsol soils of Quebec, certain characteristics of which have been described in previous papers (1, 2).

The soils from which isolations were attempted have been designated by the letters used previously. In addition, isolations were attempted from the horizons of a heavily leached lowland podsol soil, under mixed coppice (deciduous) near St. Michel de Rougement, Rouville County, Que.; this soil, sampled on June 20, 1933, has been designated as MR.

In regard to the organisms isolated from any horizon, it must be recognized that the presence of any organism in the lower layers (horizons) of soil may be due solely to the movement of water. This may be particularly important in relation to the bacteria utilizing soluble material derived from the organic matter. It must also be recognized that it is not possible to obtain a sample from one horizon without some admixture of insoluble material from a contiguous horizon. The survival of an organism may, however, be dependent upon the conditions in the soil material, the bulk of which gives an environment similar to natural conditions. The samples were kept in closed containers that did not permit excessive loss of moisture; the conditions in the samples thus resembled the natural condition, so that the organisms isolated would probably prove to be those capable of active life in the soil.

In all of the isolations that were attempted, with the exception of those for bacteria decomposing urea, the same basic mineral medium was used, to which were added sources of carbon or nitrogen as required. The composition of the mineral salts medium was as follows:

K_2HPO_4 0.1%; $MgSO_4.7H_2O$ 0.01%; NaCl 0.02%; $CaCl_2$ 0.01%; $FeCl_3.6H_2O$ 0.002%.

A search was made for bacteria belonging to the following groups that are of biochemical importance in agriculture.

1. Bacteria decomposing cellulose.
2. Bacteria decomposing benzene-ring compounds.
3. Nitrogen-fixing bacteria.
4. Bacteria decomposing urea.

Cellulose Decomposition

Soils of 1931

Two grams of moist soil from each horizon of soils R and S were added to 25 ml. of mineral salts medium in Erlenmeyer flasks of 125 ml. capacity, in three series; to one was added 0.2% ammonium sulphate, to another was added 0.2% sodium nitrate; to the third no nitrogen was added. A sterile filter paper, Whatman No. 41, 11.5 cm. in diameter, was folded to admit the wide end into a flask, so that it remained upright in the medium, and thus provided a large area of surface for aerobic organisms. On a basis of dry soil, the amounts of inoculum were in most cases more than one gram. The cultures were incubated at 26° C. on July 12, 1932. After 17 days the cultures were examined; with one exception, the organisms found on the cellulose were fungi.

The inoculum from horizon B of soil R developed a growth of *Cytophaga hutchinsoni*, together with associated bacteria, in the medium containing sodium nitrate. The results are shown in Table I.

TABLE I

ORGANISMS DEVELOPING IN CELLULOSE MEDIUMS
(Soils of 1931)

Soil	Horizon	Nitrogen added to medium		
			Sodium nitrate	Amonium sulphate
R	A_1	Fungus	Fungus	Fungi
	A_2	No growth	Fungus	Fungus
	B	No growth	*Cytophaga et al.*	Fungus
S	A_1	Fungus	Fungi	Fungi
	A_2	No growth	No growth	Fungi
	B	No growth	No growth	Fungus

Conditions in these horizons were evidently unsuited, with the exception noted, for the viability of bacteria commonly found in agricultural soils and capable of growth in the mediums used. It is possible that the addition of the mass of soil, from 0.62 to 1.7 gm. on the oven-dry basis, so altered the conditions in a medium as to render it unsuitable for the bacteria.

Soils of 1933

Approximately 1-gm. samples of fresh moist soil from the separate horizons of soils LM, VC and MR, were inoculated into cellulose-salts medium (June, 1933); in the case of the organic matter horizons, 1 : 10 dilutions were also inoculated. Cultures were examined at intervals for a month, and cellulose-destroying bacteria were found in two organic matter horizons (A_1) and in one illuvial horizon (B_2). The results are shown in Table II.

The bacterial decomposition was wholly that of the organisms mentioned; no *Vibrio* spp. were noted, whose characteristic action is usually shown by

disintegration of the fibres of the filter paper. The other forms noted as present were usually short rods, commonly found as contaminants in *Cytophaga* cultures.

TABLE II

ORGANISMS DEVELOPING IN CELLULOSE MEDIUMS

(Soils of 1933)

Soil	Horizon	Organisms present after		Remarks
		15 days	30 days	
LM	A_1	No growth	Black slimy growth	No fungi; no bacteria isolated.
	A_1 (1 : 10)	*Micrococcus*	*Micrococcus*	Not isolated.
	A_2	No growth	No growth	—
	B_1	No growth	No growth	—
	B_2	*Cytophaga*	*Cytophaga*	Other forms present.
VC	A_1	Yellow growth	Fungi; black growth	No *Cytophaga*.
	A_1 (1 : 10)	No growth	Slimy	—
	A_2	No growth	Gray and brown fungi	—
	B	No growth	Fungus	—
MR	A_1	*Cytophaga*	—	—
	A_1 (1 : 10)	Yellow growth and fungi	—	Not isolated.
	A_2	Yellow growth	—	*Cytophaga* and other forms.
	B	No growth	—	—

In a later test (October 31, 1933) small quantities of soil from horizons of VC and MR were inoculated into test tubes of medium, cellulose being added in the form of a strip of filter paper. *Cytophaga sp.* was found again in the organic matter horizon of soil MR, but did not develop from soil VC. The organic matter (A_1) horizon of the latter soil consisted of spruce raw humus. The pH of the samples, after storage, varied from 4.00 to 4.67.

Bacteria Decomposing Benzene-ring Compounds

Soil from the horizons of the lowland podsol MR, and of the "coniferous" podsol, VC, was inoculated into a sterile medium composed of mineral salts, ammonium sulphate, and either phenol, 0.05%, or naphthalene crystals previously sterilized in alcohol. The soils had been stored for four months in a closed container before the cultures were made. In these tests, positive proof of an organism's capacity to decompose the carbon compound supplied was accepted only after growth had become well developed in sub-cultures.

When phenol was used in the selective medium, cultures belonging to the genus *Mycobacterium* were isolated from the organic matter horizon and from the B horizon of the lowland podsol soil MR. In pure culture these organisms attacked phenol, but not naphthalene. The cultures from the horizons of

the "coniferous" Laurentian podsol VC proved sterile. When naphthalene was used in the selective medium, organisms of the genus *Mycobacterium* were found to be present in all of the horizons of soil VC, and in the B horizon of soil MR. The mycobacteria isolated from the B horizons, after transfer and plating in beef-peptone agar, were found capable of attacking phenol, but not naphthalene; those isolated from the eluvial (A_1, A_2) horizons of the "coniferous" soil attacked naphthalene and phenol, but appeared later to have lost the ability to decompose the former compound.

The bacteria were classified as belonging to the genus *Mycobacterium* on the basis of their resemblance in morphology, and in growth on nutrient media, to similar organisms previously isolated by means of selective media containing the same sources of carbon (3).

Nitrogen-fixing Bacteria

Attempts were made to isolate aerobic bacteria by means of nitrogen-free mediums containing mannitol or dextrine, and anaerobic bacteria with the same basic medium containing dextrose.

Soils of 1931

About 1 gm. of air-dried soil from each horizon of soils R and S was added (March 8, 1932) to 20 ml. of medium in Erlenmeyer flasks of 125 ml. capacity; the medium contained 1% of mannitol or of dextrine, and 0.5% of calcium carbonate. The cultures were incubated at 27° C. After nine days gas was visible in all of the cultures, and an examination of films showed that *Azotobacter* was absent from all. This was confirmed by plating on dextrine agar. Three months later the soils were inoculated again into mannitol medium, and *Azotobacter* again found to be absent. A summary of the results of this later test is given in Table III.

TABLE III

MICRO-ORGANISMS FOUND IN NITROGEN-FREE MANNITOL CULTURES

(Samples from soil horizons of 1931)

Soil	Horizon	Gas	Organisms after 5 days	Cultural character after 17 days
R	A_1	Present	Short rods, long rods, spores.	Moulds
	A_2	Present	Long rods	Moulds
	B	Present	Cocci, spores	No change.
S	A_1	Present	Long rods, spores, *B. amylobacter*	Moulds.
	A_2	None	Long rods	No gas.
	B	Present	Cocci, rods, spores	No change.

Soils of 1933

One-gram samples from the separate horizons of soils LM, VC, and MR were added (June, 1933) to mannitol medium. *Azotobacter* was not found in any of these horizons; the presence of gas indicated the prevalence of anaerobic

bacteria. Cells resembling the organism *Bacillus amylobacter*, and other forms, were found by direct observation of wet-mount films. An attempt was made, therefore, to determine the morphology of the anaerobic nitrogen-fixing bacteria in the horizons of the soils sampled in 1933. For this purpose a medium containing 1% of dextrose was prepared in test tubes, and cultures prepared anaerobically by means of platinized asbestos. The mineral-salts medium in double strength and 2% dextrose solution were sterilized separately and mixed just before inoculation with a small mass of soil. Before incubation, the test tubes containing the inoculum were heated to 80° C. for 10 min. They were then cooled rapidly and the mouth closed with the rubber stopper bearing the heated platinized asbestos; hydrogen was passed through for two minutes, and all connections sealed with gold size.

After a short time of incubation at 20° C., cultures from all of the horizons developed gas, a cloudy white growth becoming a viscid or a granular sediment, and an odor of butyric acid. Microscopic observation showed that organisms, referable to the morphological type *Bacillus amylobacter*, were present in large numbers, the spores characteristically swelling the cells or situated terminally. After transference to fresh medium the same characteristic growths developed, and in slanted agar of the same composition clostridium forms were found after staining. The isolation of a pure culture was not attempted, except in the case of the culture from horizon B_1 of soil LM, for the combination of heating with the selective medium allowed but few contaminants to grow.

Bacteria Decomposing Urea

The selective medium used for the development of urea-decomposing bacteria consisted of urea 2%, dipotassium hydrogen phosphate 0.05%, in distilled water. Small masses, less than 1 gm., of material from each horizon of soils LM, VC and MR were added to the medium, and incubated at 26° C. (October 28, 1933).

The presence of urea-decomposing bacteria was indicated by a strong odor of free ammonia. Transfers were then made to test tubes of urea medium, containing 5% beef extract as a source of carbon. After incubation for a few days at 26° C., the presence of ammonia was confirmed by testing with Nessler's reagent in a porcelain plate; cultures that gave a positive test were plated on urea beef-extract gelatine. Bacteria able to split urea in pure culture were isolated from all horizons of the two Laurentian soils, LM and VC, but not from the lowland soil MR. The bacteria were found to be Gram-negative spore-forming rods, growing well on urea beef-extract gelatine, but poorly on beef-peptone agar. Further studies are needed to determine their systematic position.

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STUDIES OF TOTAL ERYTHROCYTE AND LEUCOCYTE COUNTS OF FOWLS

III. VARIATION IN NUMBER OF BLOOD CELLS OF NORMAL FOWL[1]

BY JACOB BIELY[2] AND E. I. PALMER[3]

Abstract

Following the technique recommended by Shaw in which the erythrocytes and leucocytes are counted in the same chamber, the authors made total erythrocyte and leucocyte counts of 100 apparently healthy and vigorous single-comb White Leghorn hens. Following the counts, post-mortem examinations did not reveal any striking abnormalities. With few exceptions the birds were singularly free from intestinal parasites.

The range of the erythrocyte counts was found to be 1,805,000 to 3,845,000, and of leucocyte counts 18,330 to 49,000. The data are analyzed statistically.

In respect to the erythrocyte counts, the authors' data are in complete agreement with the recent studies by Cook and Dearstyne of 75 female birds. There is, however, a marked difference in the range (3,000-47,000) and the mean of the leucocyte counts, which may be due to the different technique employed by Cooke and Dearstyne.

Total erythrocyte and leucocyte counts were also made of 47 one- to two-day-old chicks. The range of the erythrocyte counts was found to be 1,800,000 to 3,180,000 and of the leucocyte counts 15,000 to 28,330. A statistical analysis of the data is presented. In the case of the *baby chicks*, since the environmental conditions are relatively uniform, it would appear that factors other than environment affect the total erythrocyte and leucocyte counts of individual chicks.

Considerable variation has been noted in the total count of red and white cells of the blood of normal fowls by different investigators. Comparatively little consideration, however, seems to have been given to the influence of such factors as breed, sex, age, diet, climate and geographical habitat, on the numerical constituents of the blood. Moreover, a systematic study of the extent and degree of the variation, as in the case of other domestic or laboratory animals, has not been reported.

Table I shows a comparison of erythrocyte and leucocyte counts reported in the literature. A part of the data was taken from the excellent review by Scarborough (21) of all available recorded findings up to 1931 on the haematology of the fowl.

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Scarborough's data, as well as additional data reviewed by the authors, are listed in Table I. Scarborough states that the average number of erythrocytes found in 246 counts by 20 investigators is 3,440,000 per cu. mm., with a normal range of from about 2,800,000 to 4,500,000. The average

TABLE I

TOTAL ERYTHROCYTE AND LEUCOCYTE COUNTS OF NORMAL FOWLS AS DETERMINED BY DIFFERENT INVESTIGATORS

Author	Year	Erythrocytes			Leucocytes		
		Ave.	Max.	Min.	Ave.	Max.	Min.
Stoltzing (21)	1856	3.9					
Malassez (21)	1872	3.1					
		2.7					
Hayem (21)	1889	2.4			26.3		
Albertoni and Mazzoni (21)	1891	2.5			32.3		
Moore (21)	1895–6	3.6	3.7	3.5	20.0	21.2	18.9
Heinz (21)	1901	4.0	4.1	3.9			
Ward (21)	1904	3.3	4.0	2.4	36.2	24.0	61.0
Warthin (21)	1907	2.5	3.0	2.0	21.0	29.0	12.0
Ellerman and Bang (21)	1908	3.0			30.0		
Hirschfeld (21)	1909	3.0			30.0		
Goodall (21)	1910	3.2			19.0		
Hedfeld (21)	1911	4.2	5.1	3.6	24.0		
Klieneberger and Carl (21)	1912	3.1				60.0	35.0
Schmeisser (21)	1915	3.5	4.0	3.0		80.0	20.0
Pickens (20)	1915–16	3.5	4.0	3.0	35.0		
Taylor (21)	1916				23.7	30.0	17.0
Fatham (21)		4.7	5.8	3.6			
Launoy and Levy-Bruhl (21)		2.98			28.0	35.0	20.0
Mack (21)		3.0			33.8		
					55.2		
Burnett (21)	1917	3.0	4.0	2.0	25.0	36.0	18.0
					17.9		
Kaupp (12)	1918	3.–4.0			28.–35.0		
Salomon (21)	1919	3.2	3.4	3.1	38.5	49.0	28.0
					49.0	57.0	41.0
Fritsch (21)	1920	3.0					
Klieneberger (14)		M 3.4–3.9			23–32.0		
		F 2.9–3.9			23–36.5		
Kennett (13)	1926	2.9			33.0		
Chaudhuri (6)	1926	M 4.6	5.2	3.9			
		F 3.1	3.8	2.0			
Blacher (1)	1926	M 3.8	4.4	3.0			
		F 2.9	3.7	2.1			
Hayden (9)	1927–8	2.8	3.7	2.3	38.9	48.0	22.0
Breusch (4)	1928	3.5			33.3		
Blain (3)	1928				18.6	29.5	10.1
						85.0	
Kyes (15)	1929					13.0	8.0
Forkner (11)	1929	3.3	4.6	2.3	24.6	74.0	6.8
Thomsen and Engelbreth Holm (24)	1931	3.0			30.000		
Fenstermacher (10)	1932	2.6	2.8	2.2	2.4	29.3	19.8
Seagar (22)	1933	2.9			27.0		
Cook and Dearstyne (7)	1934	M 3.9	4.2	3.7	14.7	19.1	9.3
		F 2.8	3.5	1.8	16.8	47.0	3.0
Landauer and David (16)	1933	M 4.3	4.6	4.0			
		F 3.4	3.7	3.2			
Gibbs (8)	1934	3.2			24.0		
Blakemore (2)	1934	2796			25.1	36.0	13.3

number of leucocytes found in 126 counts by 13 investigators is 25,900 per cu. mm. with a normal range of from about 20,000 to 40,000. The averages as given by Scarborough do not take into account the sex, age, weight, environmental conditions or geographic locations of the fowl.

It will be seen from the data presented by Scarborough that the observations of the individual authors were based on a comparatively small number of birds. A more extensive study of the haematology of the normal fowl, based on 80 normal individuals, was reported recently by Cook and Dearstyne (7).

Forkner (11) states that "an analysis of the literature reveals that most of the discrepancies in the blood counts of normal birds as given by various investigators are due to four causes. These are: study of insufficient numbers of animals; failure to make a sufficient number of observations on single animals; lack of an adequate method of counting blood cells; and confusion of thrombocytes with other elements of the blood. It must also be borne in mind that there are large individual fluctuations of the leucocyte counts of fowls which are considerably greater than those encountered in mammals."

In passing, it may be noted that up to 1928 the estimation of the number of leucocytes in the blood of chickens was based on an indirect count in which the ratio of white cells to red cells was calculated from fixed smears. Investigations conducted since 1928 for the most part are based on a direct method of counting red and white cells as well as thrombocytes.

The purpose of the present paper is to report data on the total erythrocyte and leucocyte counts of a uniform group of normal birds.

Material and Methods

The birds used in this investigation were 100 S.C. White Leghorn females. With the exception of a few individuals they were not in laying condition, since they had just completed their first egg laying year and were approximately 20 months of age. The 100 females were secured from different farms and kept together for about one month in one laying house before the blood counts were begun. Mash, grain and water were before the birds at all times.

Owing to the season of the year (October-November) the birds were moulting, some being in an early, while others were in an advanced stage of the moult. Throughout the period of observation the birds appeared to be healthy and vigorous.

The method and technique adopted in making the total erythrocyte and leucocyte counts were those described previously (17, 18). The method was that recommended by Shaw (23). An attempt was made to maintain conditions that were as nearly uniform as possible. Blood dilutions of the first 40 birds were made with one pipette, while the blood of the remaining 60 birds was diluted with another pipette. The same counting chamber was used for all the counts.

A record was made of the room temperature, the time of day the count was made, the weight of the bird, and the number of red and white blood cells counted on each side of the counting chamber. The counts of the 100 birds were completed within about three weeks. Total erythrocyte and leucocyte counts were made also of 47 one- to two-day-old S.C. White Leghorn chicks later in the season.

TABLE II

ERYTHROCYTE AND LEUCOCYTE COUNTS OF 100 S.C. WHITE LEGHORN HENS

No. of Bird	Erythrocytes (000)	Leucocytes	No. of Bird	Erythrocytes (000)	Leucocytes
1	3,175	18,880	51	2,425	38,440
2	2,145	24,550	52	2,490	44,770
3	2,925	29,770	53	2,630	34,880
4	2,985	29,770	54	2,600	34,220
5	3,260	34,880	55	2,730	36,330
6	3,310	34,330	56	2,470	26,440
7	2,615	18,330	57	2,705	35,550
8	3,235	34,000	58	2,750	32,220
9	2,850	35,880	59	2,710	36,000
10	2,800	36,000	60	2,725	32,110
11	2,585	40,660	61	2,945	37,550
12	2,560	30,660	62	2,615	30,220
13	2,550	19,000	63	2,475	22,550
14	2,725	46,220	64	2,505	24,440
15	3,155	32,330	65	2,980	30,220
16	2,955	43,000	66	2,405	28,000
17	2,765	30,000	67	2,530	25,880
18	2,775	38,440	68	2,315	30,880
19	2,685	34,000	69	3,415	38,660
20	3,845	35,880	70	2,590	23,000
21	3,205	25,330	71	2,315	30,110
22	2,305	39,880	72	3,210	49,000
23	3,110	37,880	73	2,760	29,330
24	2,880	31,880	74	2,570	40,110
25	2,770	23,440	75	3,025	33,000
26	2,780	32,770	76	2,390	34,660
27	3,160	32,330	77	2,600	30,330
28	2,920	22,880	78	3,340	20,330
29	2,715	22,550	79	2,575	33,220
30	3,490	30,770	80	2,800	39,660
31	3,570	35,000	81	2,990	29,000
32	2,375	20,000	82	2,930	35,770
33	2,885	34,000	83	2,450	23,880
34	3,020	37,220	84	2,140	40,440
35	2,905	36,660	85	2,975	30,660
36	2,730	29,220	86	3,265	47,000
37	2,675	34,440	87	2,800	33,880
38	2,435	34,330	88	2,645	23,000
39	2,505	41,000	89	2,925	31,440
40	2,690	33,110	90	2,810	33,880
41	2,395	31,550	91	3,050	21,550
42	2,820	38,000	92	2,880	29,440
43	3,055	29,660	93	2,545	34,110
44	2,685	28,550	94	3,010	33,000
45	2,760	32,550	95	3,070	23,660
46	3,130	25,660	96	2,575	25,110
47	2,485	26,220	97	2,775	41,880
48	2,425	38,880	98	3,060	32,330
49	1,805	25,000	99	3,365	45,770
50	2,475	32,000	100	3,250	26,440

Data

Detailed data pertaining to the erythrocyte and leucocyte counts of 100 single-comb White Leghorn females are shown in Table II. It will be seen that the mean erythrocyte count was 2,782,000 per cu. mm. and the mean leucocyte count was 32,150 per cu. mm. The range in the erythrocyte counts was 1,805,000 to 3,845,000 and that of the leucocytes 18,330 to 49,000.

Tables III and IV show the frequency distribution of the erythrocyte and leucocyte counts respectively. This is strikingly illustrated in Figs. 1 and 2.

TABLE III

FREQUENCY DISTRIBUTION OF THE ERYTHROCYTE COUNTS OF 100 S.C. WHITE LEGHORN HENS

No. of erythrocytes (000)	Under 1999	2000–2249	2250–2499	2500–2749	2750–2999	3000–3249	3250–3499	3500–3749	3750–3999
Percent. of total birds	1	2		28	28	15	8	1	1

TABLE IV

FREQUENCY DISTRIBUTION OF THE LEUCOCYTE COUNTS OF 100 S.C. WHITE LEGHORN HENS

No. of leucocytes	Under 19,999	20,000–24,999	25,000–29,999	30,000–34,999	35,000–39,999	40,000–44,999	45,000–49,999
Percent. of total birds	3	13	17	37	19	7	4

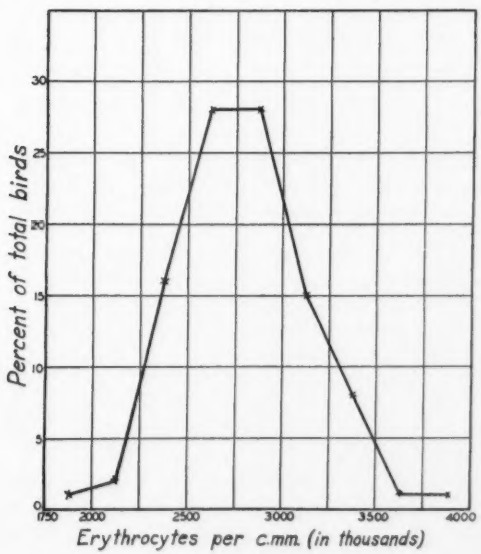

FIG. 1

TABLE V

STATISTICAL ANALYSIS OF ERYTHROCYTE AND LEUCOCYTE COUNTS

	Authors' data on 100 ♀ birds		Cook and Dearstyne data on 75 ♀ birds		Authors' data on 1- to 2-day-old chicks	
	Erythrocytes	Leucocytes	Erythrocytes	Leucocytes	Erythrocytes	Leucocytes
Mean	2782000 ± 22580	32150 ± 450	2760000 ± 26578	16800 ± 726	2480000 ± 31576	20600 ± 286
Standard deviation	334777 ± 15969	6680 ± 318	338885 ± 18735	9265 ± 509	318338 ± 22366	2889 ± 202
Coefficient of variability	12.03 ± 0.58	20.77 ± 1.02	12.27 ± 0.67	55.09 ± 3.80	12.83 ± 0.91	14.02 ± 0.99

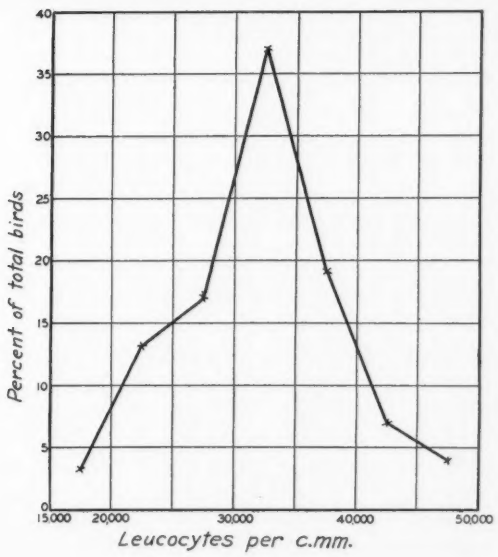

FIG. 2

The data presented in Table II were analyzed statistically by the usually accepted formulas (17). Determination of the following numerical values for the total red and white cells was made: mean counts, the probable error of the mean, standard deviation and its probable error, coefficient of variation and its probable error. (Table V).

The average number of red cells counted on each side of the chamber was 281.24 and 279.14 or a difference of 2.10 cells and of white cells 142.41 and 145.15 or a difference of 2.74 cells. The difference between the number of cells counted on both sides of the chamber, according to class ranges is shown in Table VI. In the great majority of cases there was very little difference

TABLE VI

DIFFERENCE IN THE NUMBER OF CELLS COUNTED ON BOTH SIDES OF THE CHAMBER

Range in number of cells	0–5	6–10	11–15	16–20	21–25	26–30
Erythrocytes, No. of birds	26	40	26	7	1	
Leucocytes, No. of birds	34	32	26	2	2	4

in the number of cells counted on both sides of the chamber, indicating that the actual counting was performed with great accuracy. As a matter of fact the actual difference was negligible since in each case the average of the two counts was taken.

The erythrocyte and leucocyte counts of the 47 one- to two-day-old chicks are shown in detail in Table VII. The mean erythrocyte count was 2,480,000 per cu. mm. and the mean leucocyte count was 20,600 per cu. mm. The

TABLE VII

ERYTHROCYTE AND LEUCOCYTE COUNTS OF 47 ONE- TO TWO-DAY-OLD S.C. WHITE LEGHORN CHICKS

No. of bird	Erythrocyte (000)	Leucocyte	No. of bird	Erythrocyte (000)	Leucocyte
1	2885	18,770	25	2030	17,440
2	2580	21,550	26	2525	23,220
3	2745	17,440	27	2830	18,770
4	3050	20,110	28	2540	22,110
5	2625	23,000	29	2595	17,000
6	2030	15,000	30	2175	15,440
7	2480	23,440	31	2265	16,220
8	2845	23,550	32	2315	19,880
9	3180	22,000	33	2430	18,000
10	2515	25,110	34	2265	22,880
11	2635	15,220	35	2345	20,550
12	2590	22,440	36	2695	22,220
13	2390	21,660	37	2265	23,660
14	2170	19,550	38	2955	15,000
15	2900	22,660	39	2250	15,660
16	2230	23,550	40	2045	17,770
17	1800	19,550	41	2110	28,330
18	2835	20,330	42	2065	22,880
19	2710	23,110	43	2410	24,660
20	2545	16,440	44	2320	22,330
21	2010	15,000	45	1910	20,550
22	2095	20,880	46	2980	15,330
23	2610	23,110	47	2365	19,880
24	2240	21,770			

range in the erythrocyte counts was 1,800,000 to 3,180,000 per cu. mm. and in the leucocyte counts 15,000 to 28,300 per cu. mm. A statistical analysis of the data is shown in Table V.

Considerable difficulty was at first experienced in obtaining accurate blood cell counts of baby chicks. Extreme care had to be exercised in securing the blood and mixing it immediately with the diluting fluids.

Discussion

The first problem that confronted the authors in this investigation was the question of the "normality" of the birds. Observations based on physical appearances indicate that so-called normal birds may show various degrees of internal pathological involvement. Suffice it to recall that pullorum disease "carriers" which appear to be normal nearly always show a diseased condition of the ovary and sometimes of the heart.

In order to gain some idea about the normality of the 100 birds used in this investigation, every bird was killed and a detailed post-mortem examination carried out. The condition of the intestine was carefully examined and a record made of any coccidia or other intestinal parasites found.

With few exceptions the internal organs appeared to be normal. Two birds were found with only one kidney each. The intestines were singularly free from parasites and no coccidia could be detected in the scrapings of the intestinal tract. The liver, spleen and kidneys of each bird were weighed and with few exceptions appeared to be normal in size, texture, and color. All other organs appeared normal. The healthy condition of the birds may be attributed to the fact that only the most vigorous birds had been retained as a result of rigorous culling of the flock during the laying year. A heavy infestation of round worms and tape worms was found in two birds. In the case of a few birds the ovary showed slight pathological involvement and *S. pullorum* was isolated. It may be noted that in these cases the erythrocyte and leucocyte counts did not deviate from the average counts of the whole group.

It may be assumed from the post-mortem examination that the birds used in this investigation were as near normal as could be expected of birds kept under ordinary commercial conditions. As far as the authors are aware, this study is the only one in which examination of the internal organs was made immediately following the taking of the blood counts.

The data presented in this investigation fall within the range of the minimum and maximum values reported by previous investigators. An examination of Figs 1 and 2 and Tables III and IV shows that the frequency distribution of the erythrocyte and leucocyte counts approached a normal curve. It is significant that the probable error of the mean, of the standard deviation and of the coefficient of variation is in each case low.

The coefficient of variability which represents an absolute measure of variability is in the case of the erythrocytes 20.77 ± 1.02 and in the leucocytes 12.03 ± 0.58. It may be noted that in a statistical study of the variation in the blood counts of 100 normal rabbits, Bushnell and Bangs (5) reported a coefficient of variation of 13.01 in the erythrocyte and 20.83 in the leucocyte. Similarly Pearce and Casey (19) showed that the coefficients of variation of erythrocytes and leucocytes, based on 1110 counts of 174 normal rabbits, were 12.09 and 20.53 respectively.

Since the studies of Cook and Dearstyne (7) are the only ones based on a large number of birds, comparable in numbers to ours, it seems worth while to compare our results with theirs. To facilitate analysis the data of both investigations are shown in Table V.

It will be seen that the mean, minimum and maximum of the erythrocyte counts reported by Cook and Dearstyne correspond closely with those reported in this investigation. The frequency distribution in the different classes is practically the same.

With regard to the total leucocyte counts there appears to be considerable disagreement between the data presented in this paper and those of Cook and Dearstyne. (Range 3000–47,000). A statistical analysis made of the data pre sented by Cook and Dearstyne (Table V) shows that the coefficient of variability of the erythrocytes was comparatively low while that of the leucocytes was very high.

The wide divergence found between the leucocyte counts reported by Dearstyne and by the writers may be due to differences in technique or to the physiological condition of the birds employed in the two investigations. The high coefficient of variability (55.09 ± 3.80) of the leucocyte counts of Cooke and Dearstyne's birds suggests that their group of birds may not have been as uniform physiologically as the present series (coefficient of variability 20.77 ± 1.02). As regards technique, that used by the authors was Shaw's (23) while that used by Cook and Dearstyne was Forkner's (11). In the latter technique, both the direct and indirect method are involved, while in the former, the direct method only is used.

It would seem from a consideration of the data presented in this investigation that no true comparison can be made between the results of one investigation and those of another, until more uniform methods of making total leucocyte counts are adopted.

The data show that considerable variation has been found in the erythrocyte and leucocyte counts of a fairly uniform group of birds such as recorded in this paper. The variation in the leucocyte counts was greater than that of the erythrocyte counts. It is of interest to note that the mean erythrocyte and leucocyte count of the one- to two-day-old chicks was lower than that of the adult birds and that the difference was statistically significant. The standard deviation and coefficient of variability were much lower in the one- to two-day-old chicks than in the adult birds. In the case of the baby chicks, since the environmental conditions were relatively uniform it would appear that factors other than environment may affect the total erythrocyte and leucocyte counts of individual chicks.

Aside from the question of standardized technique and uniformity of the subjects under investigation, there may be genetic factors that influence the total red and white count of various families or strains of birds kept under the same conditions. A study of the genetic aspects of the question might, in part, account for some of the variations noted in this and other investigations.

Acknowledgment

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THE WHITE APPLE LEAFHOPPER (*TYPHLOCYBA POMARIA* McA.)[1]

By Thomas Armstrong[2]

Abstract

Typhlocyba pomaria McA., the white apple leafhopper, a pest of apple in Canada and the United States, has two generations a year in the Vineland district of Ontario. The insect spends the winter in the egg stage, the newly hatched nymphs make their appearance in May, and adults are present on the trees in June and July. Summer eggs are deposited chiefly in the midribs of the foliage, and take from 20 to 64 days to hatch. The second generation of adults matures in August and September. Detailed information on all phases of the life history is presented, including the type of feeding and nature of the injury produced on apple foliage. The associated species of leafhoppers which cause injury to apple are listed.

Introduction

The species *Typhlocyba pomaria* McA., until 1926, was confused with other species of leafhoppers attacking apple. In the main the insect has been taken for *Typhlocyba rosae* (L.) in American and Canadian literature, and it is altogether likely that publications dealing with the rose leafhopper as an apple pest, before 1927, may apply, in part at least, to *T. pomaria*. McAtee (12) first differentiated the two species in 1926, and gave a description of *T. pomaria*. Ackerman (1), in 1931, proposed the common name "white apple leafhopper" for this apple pest, and this name was approved by the American Association of Economic Entomologists.

In 1931, DeLong (6) wrote the following with regard to the confused status of the leafhoppers attacking apple. "*T. pomaria* is the most common and wide-spread species of this genus occurring on apple. It is apparent that previous records of *rosae* in abundance are in error, and observations in areas where *rosae* was previously reported have shown either *pomaria* or *xanthippe* to be the important species. The species reported by Childs (5) as *rosae* is apparently *pomaria* since that is the important species of *Typhlocyba* occurring in that area (Oregon) according to recent observations."

T. pomaria has been present in Ontario apple orchards for a considerable number of years. In 1919 it was reported (14) that myriads of leafhoppers were present in many apple orchards in the Niagara district, the species at that time being called *Empoa rosae*. Mr. W. A. Ross* is of the opinion that this species was really *pomaria*. It would appear that there was a bad infestation in Ontario during the seasons of 1919 and 1920, followed in 1921 by a considerable reduction in leafhopper population brought about by natural agencies. In 1927, the white apple leafhopper was very abundant, especially in the counties of Prince Edward, Northumberland, Durham, Peel, Went-

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worth, Lincoln and Norfolk. It was sufficiently abundant in 1931, in the Niagara district and western Ontario, to cause conspicuous mottling of the foliage late in the season, and in 1932 some orchards were very heavily infested. Mr. Ross reports the species as occurring in British Columbia, and Gilliatt (14) records it as present in Nova Scotia apple orchards, and being particularly numerous in 1930 in the Berwick district. It was prevalent and widespread in Annapolis Valley orchards during 1931.

In the United States the species is widely distributed, being known to occur in the states of New York, Connecticut, Pennsylvania, Virginia, Arkansas, Oregon and Idaho. In all probability *T. pomaria* will be found in most of the apple growing districts.

The information contained in this paper was compiled from original notes taken during the years 1933 and 1934. The work was carried out at Vineland Station, Ontario.

The First Generation

Overwintering Egg

Description

The egg is white, elongate, cylindrical and slightly tapering at the ends, which are quite blunt. The anterior end, that portion deposited last, has a noticeable curve upwards to the surface, tending to form a channel. The average length of the egg is .80 mm., and the width, .26 mm.

Location

The overwintering eggs are laid singly beneath the bark in the collenchyma tissue of the cortex. In most cases they are situated crosswise to the axis of the stem, and appear to the eye as raised areas, or small blisters, quite regular in outline. The egg itself lies practically horizontal, with possibly a slight slope down towards the posterior end.

Distribution on the Apple Limb

An apple limb was examined on April 19, 1933, to see where the leafhopper eggs were laid. Table I gives the results.

TABLE I

Year wood formed	Length of growth, in.	Number of fresh eggs	Remarks
1932	9	0	—
1931	8	5	—
1930	13	45	—
1929	5	53	Numerous old egg blisters
1928	4	52	Numerous old egg blisters
1927	5	49	Numerous old egg blisters
1926	4	23	Numerous old egg blisters
1925		Fresh eggs present	
1924	—		Numerous old egg blisters

Process of Hatching

When hatching takes place the "head" end of the slightly concave side of the blister shows a break in the bark tissue, from which the embryo protrudes. The nymph, as it slowly appears, breaks its protecting sheath and slides forth, leaving the egg shell thrust well out of the old egg cavity.

TABLE II

DURATION OF HATCHING OF FIRST GENERATION EGGS
(Cages in McKenzie's Orchard)

Date	Position of cage on tree				Total
	North	South	East	West	
May 9	9	5	1	3	18
10	7	14	9	17	47
11	51	42	11	21	125
12	8	14	15	7	44
13	28	21	30	24	103
14	27	22	30	25	104
15	23	41	33	31	128
16	16	17	30	19	82
17	24	22	33	18	97
18	34	18	55	29	136
19	29	14	35	11	89
20	12	5	11	4	32
21	12	4	12	4	32
22	9	7	8	5	29
23	4	4	7	3	18
24	2	2	1	2	7
25	2	2	1	2	7*
26	4	3	3	1	11*
27	3	3	4	0	10*
28	2	3	3	1	9*
29	4	4	16	2	26*
30	7	2	4	4	17*
31	4	2	2	9	17*
June 1	3	2	3	1	9*
2	0	0	2	4	6*
3	0	0	0	1	1*
4	0	0	0	0	0*
5	0	1	0	0	1*
6	0	0	0	0	0*
Totals	324	274	359	248	1205

* *E. maligna nymphs in part.*

Duration of Hatching Period

In 1933, overwintering eggs commenced hatching on May 13, at which time the apple cluster buds were separating. Newly hatched nymphs could be found in the orchards until the end of May. The following year, 1934, eggs were hatching from May 9 to June 1. Table II shows the duration of the hatching period, as well as the time when the largest number of nymphs appeared. Insectary rearing records showed that the last eggs hatched on June 1, the date on which the calyx spray was applied in the apple orchard. The percentages of the total nymphs to appear at various times during the hatching period were as follows:

May 9 First nymph
15 46.4% of eggs hatched
22 88.5% of eggs hatched
June 1 100.0% of eggs hatched

Thus two weeks after the first egg hatched practically 90 per cent of all the eggs had produced their nymphs.

THE NYMPH

Description

This insect passes through five nymphal stages, moulting five times. As moult follows moult the leafhopper gradually increases in size and the wings develop. A short description of each instar is given herewith.

First instar. Color pale white to light yellow, with eyes a dull red. The first two segments of the antennae are pale, the remainder dusky. Size, .890 mm. long, and .217 mm. wide.

Second instar. Color varies from creamy white to light yellow. Wing pads present. The posterior margin of the metathorax is definite in outline, and is the main character which separates this instar from the first and third. Eyes not as red as in first instar. Size, 1.156 mm. long, and .320 mm. wide.

Third instar. Color as in second instar. Body is stouter, with the wing pads extending to hind margin of first abdominal segment. Eyes a dull white in color. Size, 1.650 mm. long, and .400 mm. wide.

Fourth instar. Color dull white to a distinct yellowish. Eyes whitish in color. Wing pads extend to hind margin of second abdominal segment. Size, 1.970 mm. long, and .490 mm. wide.

Fifth instar. Color light yellow to a more distinct yellow. Eyes distinctly pearl white. Wing pads extend to the fourth abdominal segment. Size, 2.610 mm. long, and .686 mm. wide.

Habits

The first generation nymphs, hatching on the young wood as they do, have to wander off in search of foliage for feeding purposes. Very shortly after emergence the small ungainly creatures become very active, especially if the day is warm, and migrate rapidly along the limbs, spurs and petioles until the underside of the leaf is reached. Here, feeding is carried on. The nymphs confine their attack mainly to the lower parts of the trees, and they are never to be found on the terminal *growing* shoots. Very rarely does one find nymphs migrating, and it is the opinion of the writer that they remain on a satisfactory leaf until maturity. Only the fifth instar nymphs are capable of hopping and this they do but rarely. Even if disturbed persistently they will not always hop away, but will use their legs in the normal manner.

Duration of Nymphal Life.

Using potted apple seedlings under lantern globes in the open insectary, the length of the nymphal life, as well as the duration of each instar, was determined from 66 individuals in 1933, and 121 in 1934, as is shown in Table III.

TABLE III

Period	Maximum length in days		Minimum length in days		Average length in days	
	1933	1934	1933	1934	1933	1934
First instar	7	12	3	3	4.65	6.66
Second instar	6	9	3	3	4.10	6.16
Third instar	5	6	2	2	3.94	4.03
Fourth instar	6	7	2	2	4.40	3.95
Fifth instar	10	8	3	5	5.83	6.64
Total life	27	37	19	19	23.0	27.45

Casting of Nymphal Skin

Moulting always takes place on the underside of the leaves, and requires a period of from 15 to 30 minutes. When the time arrives, the insect sets its claws in the leaf tissue and also inserts the mouth parts. It remains quiet for a short time, and then the skin splits along the mid-dorsal line from the head to the prothorax. The nymph pushes itself forward gradually, and works its way out, leaving the old skin attached to the leaf. The first four nymphal cast skins are not fastened to the leaf very securely for they blow away readily with the lightest breeze, but that of the last moult very often remains on the foliage all summer.

When the adult leafhopper emerges from the last nymphal moult the wings rapidly expand to their full size, the outer wings developing a little faster than the under wings. The winged insect does not fly away immediately on reaching maturity but rests very quietly for several hours close to where the moulting occurred.

Time of Occurrence of First Brood Nymphs

Nymphs (Plate I Fig. 1) were present in the orchards from May 13 to June 22 (from the time the cluster buds were separating to the time when the fruits of McIntosh and Greening were slightly more than one inch in diameter), though by June 15 very few nymphs could be found, the greater proportion having attained maturity. In 1934, the nymphal population was at its peak during the first week of June.

In order to determine the nymphal population in the orchard in the spring of 1933, leaf clusters or single leaves were picked at random in equal numbers from the north, south, east and west sides of the trees, and the number of insects on this foliage was recorded. Counts were made daily from May 14 to May 20 inclusive, that is from the time of first appearance until 85% of the nymphs had hatched. The clusters chosen were from egg-bearing wood, and were thus most likely to possess nymphs. By May 19 the clusters were getting too large to examine thoroughly, so a single leaf was used. There

TABLE IV

POPULATION STUDIES OF LEAFHOPPERS, SPRING, 1933

Date	Number of clusters or leaves examined	Number of nymphs taken on the				Total nymphs	Average per cluster
		North	South	East	West		
May 14	200 clusters	97	21	78	89	285	1.43
May 15	200 clusters	209	78	75	102	464	2.32
May 16	200 clusters	42	37	28	107	214	1.07
May 17	180 clusters	143	203	240	129	715	3.97
May 18	100 clusters	202	495	110	132	939	9.39
May 19	120 leaves*	69	100	66	63	298	2.48*
May 20	100 leaves*	43	86	44	55	228	2.28*
		805	1020	641	677	3143	

* *Average per leaf. Equal to about 12 nymphs per cluster.*

would be, on the average, approximately five such leaves per cluster, so that the figures given for the counts made on May 19 and 20 should be multiplied by 5 to make them comparable with the earlier records (Table IV).

The Adult

Description

McAtee's (12) description is as follows:

"*Male.* Head and thorax pale yellow, more or less tinged with orange to reddish on front of vertex; tegmina hyaline, the costa, commissure and sectors yellow, apical cells faintly dusky; underparts pale yellowish, the face more or less tinged with orange to reddish, touches of this higher coloration elsewhere also on some specimens.

Hypopygium: Strongly distinguished by the falcate process forming the posterior border of the side of the 9th segment, which is heavily chitinized and black in colour; as the correlation of this falcate process with the type of aedeagus here illustrated for this species has thus far been constant, it results that the species can be recognized from the superficial aspect of the hypopygium, the black tips of the falces being easily seen. Near the upper posterior border of the falx are several backwardly directed bristly hairs; similar but shorter hairs form a row on the outer clasper, and longer softer hairs spring from the under side of this clasper, from the lower border of the 9th segment near articulation of outer clasper, and from the end of the anal tube. Outer claspers as seen from below depressed interiorly so that together they enclose a long narrow sunken area, clasper as a whole narrowed on posterior third, the apices somewhat spatulate and divergent; seen from side the outer clasper is curved upward at the beginning of its posterior third, in almost a right angle, apex somewhat spatulate and recurved; inner clasper slender, outcurved apically; the aedeagus as seen from above with a median and two lateral branches, the former considerably shorter than the latter, furcate at the tip, and reinforced along the lower surface by two longitudinal stripes extending nearly to the apex appearing as ribs on the back of aedeagus as seen from above; lateral processes becoming very slender apically the terminal portion curved outward and then forward. Aedeagus measured along curve, fully twice as long as connective.

Female. Paler than male, general coloration stramineous, with little or no yellow, orange, or reddish washing; tip of ovipositor sheath black. Eighth sternite moderately pointed medially and emarginate laterally.

Length, 3–3.5 mm."

Pre-oviposition Period.

The pre-oviposition period of 12 females of the first generation confined in lantern globe cages in the insectary in 1933, varied from 10 to 19 days, the average being 13.4 days. The following year, for seven females, the period averaged 9 days. The much shorter period in 1934 was due, no doubt, to

the more sunny surroundings of the open insectary where the cages rested, compared to the very sheltered conditions of the old insectary used in the 1933 work. (Table V).

TABLE V

1933			1934		
Date female matured	Date first egg laid	Pre-oviposition period, days	Date female matured	Date first egg laid	Pre-oviposition period, days
June 8	June 20	12			
9	22	13			
9	22	13			
9	25	16			
9	20	11			
9	28	19	June 9	June 18	9
9	27	18	12	21	9
10	21	11	13	21	8
10	21	11	15	25	10
12	22	10	19	29	10
12	25	13	20	28	8
12	26	14	23	July 2	9
		13.4			9

Reproductive Capacity

Records were kept of the number of viable eggs deposited by 22 females under insectary conditions. These individuals were enclosed in lantern globe cages or in small celluloid cages with a vigorous plant. One or two females were used per cage. The 22 individuals laid a total of 1174 eggs or an average of 53.4 eggs per female. Fifteen of these leafhoppers, left undisturbed throughout their oviposition period, laid an average of 62 eggs each, with a maximum of 104. In 1933, two females caged with two males when in copula on June 20, laid a total of 265 eggs or an average of 132 per female.

Period of Oviposition.

The period of oviposition varied from 7 to 34 days, as determined from the records of 15 individual females in confined quarters under insectary conditions. Egg laying extended over a period of 20.6 days on the average.

Post-oviposition Period

Female leafhoppers lived from 1 to 26 days after depositing their last fertile egg. The average post-oviposition period was 6.2 days.

Length of Life of Adults

Males survived in insectary cages anywhere from eight days to three months, the average life being 37.7 days in 1933; and 33.3 days in 1934. Females lived from 19 to 78 days, averaging 36.7 days in 1933, and 40 days in 1934. From observations in the orchard it would appear that the length of life of the insects approximates that secured under confined conditions. One leafhopper, a male, survived for 90 days.

Presence of Adults in the Orchard

The first mature apple leafhoppers were observed in the orchard on June 7, and two days later, approximately 12 days after the calyx spray, they were quite numerous. Males and females were noticed in copula for the first time on June 13. From this time until the end of July they were common in the trees. Some few could be found during the first days of August, but by this time the second generation adults had commenced to mature. Judging from the insectary rearings there is a little overlapping of the broods of adults. The last first-generation leafhopper under observation died on August 14, in 1933, and on September 7 in 1934.

For a period of two weeks starting from the time leafhoppers commenced to appear in the spring, daily orchard collections were made, and the individuals sexed. The data appear in Table VI.

TABLE VI

Date of collection	Number collected	Males	Females	Males, %	Females, %
June 6	14	14	0	100.0	0
7	28	28	0	100.0	0
8	90	90	0	100.0	0
9	56	53	3	94.6	5.4
11	84	66	18	78.5	21.5
12	61	43	18	70.5	29.5
13	74	31	43	41.9	58.1
14	63	28	35	44.5	55.5
15	72	18	54	25.0	75.0
16	88	27	61	30.7	69.3
18	78	26	52	33.3	66.7
19	60	11	49	18.3	81.7

It will be noted that during the first three days of adult emergence not a single female was collected in the orchard; and that females gradually came into the ascendency, becoming predominant in numbers one week later.

Habits of Leafhoppers

On reaching the adult stage leafhoppers are to be found resting on the under sides of the foliage. On warm days they fly very actively and, if at all abundant, can be very annoying when disturbed, for they swarm around in a cloud, and land in the hair, ears and eyes, causing some discomfort. On cool days they can be caught quite easily in vials by hand.

Mating couples were seen most frequently in the morning. The male rests with the tip of his abdomen joined to that of the female, with their heads in opposite directions, forming a straight line. The two leafhoppers remain in this position a considerable time, one pair under observation staying in copula for an hour and a half.

The Second Generation

The Egg

Location

The eggs of this generation are deposited in the under surfaces of the leaves of apple, especially along the main ribs and large veins, and quite often in the petioles. They are practically impossible to locate prior to hatching, and even after the nymph has emerged, only a tiny scar indicates where the egg was placed. If the egg is parasitized the parasite emergence hole is readily seen, and in this way the locating of the place of deposition is made quite simple.

Time of Laying

Under insectary conditions the first eggs were deposited during the third week of June,—June 20 in 1933 and June 18 in 1934—12 days after the appearance of the first leafhoppers in the orchard. In 1934, egg laying continued into August, but the majority of the eggs were laid during the month of July. A total of 2266 eggs laid in the insectary cages were deposited at the following rate:

June 18	First egg laid
30	19.6% of eggs laid
July 15	56.2% of eggs laid
31	99.0% of eggs laid

Period of Incubation

The incubation periods of 2266 eggs varied from 20 to 64 days; the average being 39 days (Table VII).

TABLE VII

Length of egg period, days	No. of eggs	Length of egg period, days	No. of eggs	Length of egg period, days	No. of eggs
20	2	35	62	50	53
21	59	36	106	51	44
22	25	37	107	52	53
23	28	38	69	53	31
24	62	39	75	54	33
25	26	40	135	55	26
26	47	41	76	56	15
27	42	42	84	57	18
28	42	43	99	58	7
29	59	44	63	59	6
30	56	45	104	60	5
31	61	46	61	61	5
32	34	47	99	62	2
33	98	48	69	63	0
34	70	49	47	64	1
					2266

In studying the periods of incubation of summer eggs it was noticed that the first eggs deposited took the longest time to hatch, and that there was a gradual scaling down in the length of the incubation period the later the eggs were laid. For instance, the 107 eggs laid on June 28–29 averaged 45.8 days for incubation, while the 47 eggs laid on July 29–30–31 only took 26.8 days. Table VIII clearly illustrates this rather unusual phenomenon. The mean daily temperature is included only to illustrate that the shortening of the period does not depend on increases in temperature.

TABLE VIII

Date eggs laid	No. of eggs	Period of incubation, days			Mean daily temp.
		Maximum	Minimum	Average	
June 20–21	46	60	37	50.8	70.5
26–27	47	61	27	46.3	70.7
28–29	107	59	25	45.8	70.9
July 4– 5	29	60	29	43.2	70.1
10–11	107	57	27	41.8	70.1
12–13	83	54	28	39.4	69.8
14–16	130	51	29	37.8	69.8
17–19	143	48	26	36.2	66.0
20–22	104	53	23	34.3	68.9
23–25	208	42	21	27.8	69.2
26–28	217	47	20	31.8	67.8
29–31	47	37	21	26.8	67.8
Aug. 1– 3	10	32	24	29.1	66.2
6– 8	9	28	22	25.1	65.8

Duration of Hatching Period

In 1933, newly hatched nymphs were found for the first time on July 17, and, according to the insectary records, the last egg hatched on September 7. In the orchard, hatching of eggs was most active during the first three weeks of August.

The duration of the hatching period was more extended in 1934. Starting on July 17, newly hatched nymphs were taken from the foliage daily until September 28, but those appearing after September 7 were few in number.

The Nymph

Duration of Instars

By rearing individuals on potted plants in the open insectary it was possible to determine the length of the five instars. The information is presented in Table IX.

TABLE IX

Period	Maximum length in days		Minimum length in days		Average length in days	
	1933	1934	1933	1934	1933	1934
First instar	8	8	2	2	4.2	3.93
Second instar	6	9	2	2	3.5	3.60
Third instar	6	9	2	2	3.8	3.92
Fourth instar	7	25	3	3	4.2	4.95
Fifth instar	9	27	4	4	6.6	7.99
Total life	29	55	17	16	22.3	23.85

Time of Occurrence

In the orchard, nymphs first appeared on July 17 and gradually increased in numbers as the season advanced. By August 2, nymphs in all five stages could be found on the foliage in small numbers. Three weeks later, August 21, they were very abundant in the McKenzie orchard. After the first week in September the nymphs rapidly decreased in quantity until by September 29 they were extremely scarce.

The Adult

Time of Occurrence

In 1933, the first leafhopper matured on August 3, in the insectary, and adults were common in the orchard by August 15, appearing in increasing numbers from this time until well into October. On October 30, after severe frosts, the following note was recorded: "Adult leafhoppers are still plentiful in the McKenzie orchard although large numbers are dead. Dead and sluggish hoppers are numerous on the soil beneath the trees. Females still seem to be actively laying as plenty were seen on the limbs with extended ovipositors as if preparing (or just concluding) to lay." Very severe frosts occurring during the first two weeks of November no doubt brought about the death of the remaining leafhoppers.

Period of Egg Deposition

Allowing for a pre-oviposition period of about 14 days it would seem that the first eggs were deposited about August 20, in 1933. Examination of apple limbs in September showed that eggs were present in the bark of wood one-half inch in diameter. In 1934, the first overwintering eggs were deposited about August 15, a few days earlier than in the preceding year.

Proportion of Sexes

Of 157 leafhoppers reared in the insectary 57% were females. From observations in the orchard, females appeared to be more abundant than males.

Feeding and Nature of Injury

The nymphs and adults of *T. pomaria* feed exclusively on the older leaves of apple and never attack the *growing* terminal shoots. They gather on the lower surfaces of the leaves, and, using their sucking mouth parts, extract the plant juices. The first indication of their presence is a slight mottling on the leaf's upper surface, and as the feeding is intensified the foliage loses its deep green color to take on a yellow-green appearance (Plate I, Fig. 2.). The white apple leafhopper does not feed on the fruit. To prove this, 80 adults were confined in a cage containing a single Wealthy apple. In four days every hopper had died, leaving the fruit without a blemish. In the same type of cage but with foliage substituted for the fruit, the leafhoppers lived for weeks.

The blemishing of the leaves and fruit by the excrement is very unsightly. (Plate I, Fig. 3). Rain removes a great deal of this spotting, but it may remain on the fruit long enough to hinder proper coloring.

PLATE I

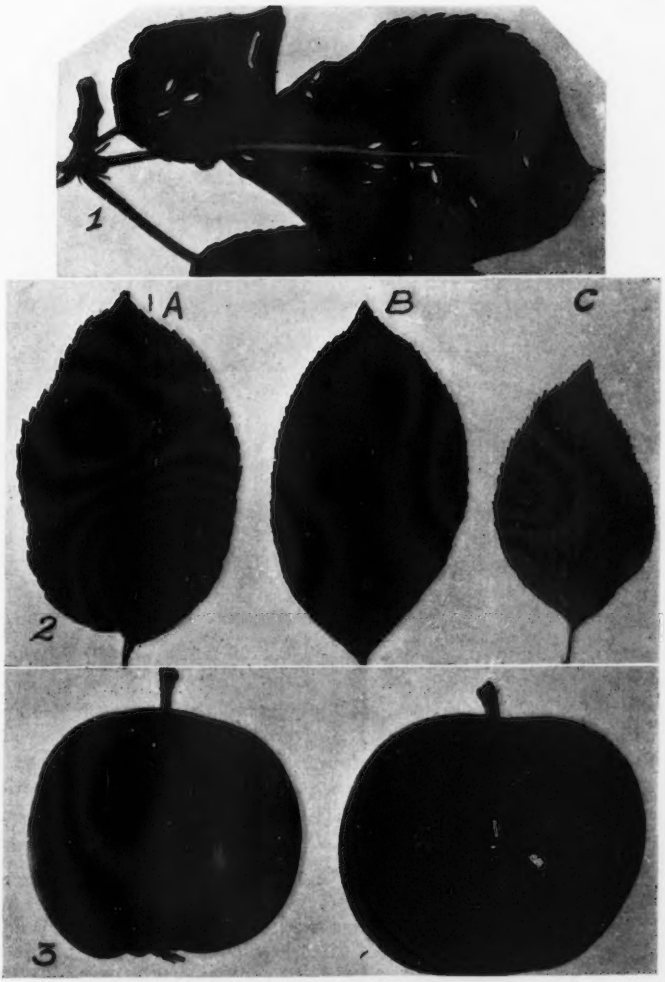
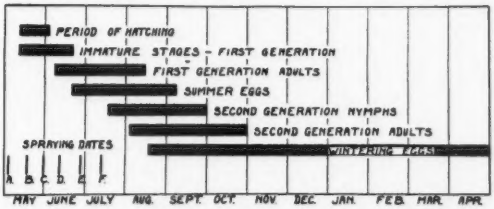


FIG. 1. *Nymphs of the white apple leafhopper feeding on apple foliage. Most of the specimens in this photograph are in the fifth instar stage.* FIG. 2. *Injury to apple foliage caused by white apple leafhoppers. A. Uninjured leaf. B. Partially injured leaf. C. Extreme injury. The small black spots in C are deposits of excrement.* FIG. 3. *Apples from a heavily infested orchard showing deposits of excrement.*

Generations

There are two generations of this insect in the year, (Text-fig 1.) with very little overlapping of any of the active stages. A few adults of the first generation are present when the early second generation leafhoppers mature. Overwintering in the egg stage, the insect first makes an appearance as a nymph in May, reaching maturity in June and July, at which time it is busy depositing


PERIOD OF HATCHING
IMMATURE STAGES - FIRST GENERATION
FIRST GENERATION ADULTS
SUMMER EGGS
SECOND GENERATION NYMPHS
SECOND GENERATION ADULTS
WINTERING EGGS
SPRAYING DATES
A B C D E F
MAY JUNE JULY AUG. SEPT. OCT. NOV DEC. JAN. FEB. MAR. APR.

TEXT FIG. 1. *The seasonal history of the white apple leafhopper, 1933. Spray dates: A. Delayed dormant; B. Pink; C. Calyx; D. Small apple; E. First cover; F. Second cover.*

eggs in the tissue of the apple leaves. The second brood of nymphs makes an appearance in July, the summer eggs taking on the average 40 days to incubate, and this in the hottest part of the season. Nymphs are numerous throughout August and most of September, and the second brood of adults can readily be found from early August to the end of October. This final brood of adults deposits eggs beneath the bark of apple limbs.

Natural Enemies

The chief predators attacking leafhoppers are spiders and birds. Adults were very commonly observed caught in the webs of spiders during the periods when flights were in progress. The predaceous Mirid, *Hyaliodes vitripennis* Say, was present in small numbers in one fairly heavily infested orchard.

Hymenopterous parasites of eggs and adults are important enemies of the white apple leafhopper. One of these, *Anagrus armatus*, var. *nigriventris* Gir., a parasite of the overwintering and summer eggs, was very common in 1933, and no doubt was instrumental in reducing the population considerably. The adult parasite, *Aphelopus* sp., nr. *microleucus* Perkins, was also abundant, more especially attacking the first brood of adults, although a small proportion of the autumn generation of leafhoppers was parasitized as well.

Associated Species of Leafhoppers

The leafhoppers which cause injury to apple in the Vineland district, in the order of their importance, are *Typhlocyba pomaria* McA., *Empoasca fabae* Harris, *Empoasca maligna* Walsh, and *Typhlocyba rosae* Linn. The grape leafhopper, *Erythroneura comes* Say, migrates to the apple in numbers in the autumn, but is not present during the greater part of the growing season. *Erythroneura lawsoniana* Baker has been taken in small numbers in a number

of apple orchards throughout the peninsula. *Empoasca fabae*, the potato leafhopper, attacks the newer growth only, curling and hopper-burning the foliage, especially on nursery stock and young trees. *Empoasca maligna* was taken on apple during the spring and summer months; this insect having one generation in the year. The rose leafhopper was present but in such small numbers that it was very difficult to find.

Acknowledgments

The leafhoppers mentioned in this paper were identified by Mr. R. H. Beamer of Kansas; the parasites, by Mr. G. S. Walley, and Mr. A. B. Gahan.

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